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Peer Reviewer of *World Journal of Methodology*, Yu-Zhuo Wang, PhD, FCAHS, Professor, Department of Urologic Sciences, University of British Columbia, 675 West 10th. Ave, Vancouver, BC V5Z 1L3, Canada. yuzhuo.wang@ubc.ca

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ORIGINAL ARTICLE

Observational Study

Pandemic and precocious puberty - a Google trends study

Athanasios Tselebis, Lina Zabuliene, Charalampos Milionis, Ioannis Ilias

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Athanasios Tselebis, Department of Psychiatry, "Sotiria" General Chest Diseases Hospital, Athens GR-11527, Greece

Lina Zabuliene, Faculty of Medicine, Vilnius University, Vilnius LT-03101, Lithuania

Charalampos Milionis, Ioannis Ilias, Department of Endocrinology, Elena Venizelou General and Maternity Hospital, Athens GR-11521, Greece

Corresponding author: Ioannis Ilias, MD, PhD, Consultant Physician-Scientist, Department of Endocrinology, Elena Venizelou General and Maternity Hospital, 2, Elena Venizelou Square, Athens GR-11521, Greece. iiliasmd@yahoo.com

Abstract

BACKGROUND

Recent publications from several countries have reported that more young people (mainly girls) are experiencing precocious puberty (PP)/menarche during the coronavirus disease 2019 pandemic compared to the past. This variation is attributed to the stress of confinement, lack of exercise, obesity and disturbed sleep patterns. A common feature of the relevant papers, however, is the small number of reported cases of PP. Studies have shown that searches for diseases on the internet also reflect to some extent the epidemiology of these diseases.

AIM

To estimate, through internet searches for PP, any changes in the epidemiology of PP.

METHODS

We assessed in Google Trends searches for 21 PP-related terms in English internationally (which practically dwarf searches in other languages), in the years 2017-2021. Additionally, we assessed local searches for selected terms, in English and local languages, in countries where a rise in PP has been reported. Searches were collected in Relative Search Volumes format and analyzed using Kendall's Tau test, with a statistical significance threshold of $P < 0.05$.

RESULTS

Internationally, searches for three PP-related terms showed no noticeable change over the study period, while searches for eight terms showed a decrease. An increase was found over time in searches for nine PP-related terms. Of the 17 searches in English and local languages, in countries where a rise in PP has been reported, 5 showed a significant increase over time.

CONCLUSION
Over the study period, more than half of the search terms showed little change or declined. The discrepancy between internet searches for PP and the reported increase in the literature is striking. It would be expected that a true increase in the incidence of PP would also be aptly reflected in Google trends. If our findings are valid, the literature may have been biased. The known secular trend of decreasing age of puberty may also have played a role.

Key Words: Data collection; Epidemiology; Puberty; Human; Methods; Trends

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Core Tip: An increasing number of young people are experiencing precocious puberty (PP) during the coronavirus disease 2019 pandemic. We estimated, through relevant internet searches, changes in the epidemiology of PP. We assessed in Google trends searches for PP-related terms in English internationally, and in English and local languages, in countries where a rise in PP has been reported, in the years 2017-2021. More than half of the search terms showed little change or declined. The discrepancy between internet searches for PP and the reported increase in the literature is striking. If our findings are valid, the literature may have been biased.

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INTRODUCTION

Precocious puberty (PP) describes the changes associated with the transition to adult body habitus earlier than age 8 in girls and age 9 in boys. Children with PP may have a short final height, suffer from poor body image, low self-esteem, depression and anxiety and/or be plagued by problems in socialization (particularly with peers), sexuality and substance abuse[1].

Recent publications from several countries[2-5] have reported that more young people[6-10] (mainly girls) are experiencing PP/menarche during the coronavirus disease 2019 (COVID-19) pandemic compared to the past[11-13]. This variation is attributed to the stress of confinement, lack of exercise, obesity, and disturbed sleep patterns. A common feature of the relevant papers, however, is the small number of reported cases of PP, with the exception of reports from South Korea (see below). Among adult internet users who look for specific health issues, approximately one in four has read or watched someone else's health experiences or medical issues in the last year; and one in six is seeking others with the same health concerns[14]. These behaviors are more prominent in caregivers and patients with chronic diseases[14]. Digital epidemiology uses digital data that were not generated with the primary goal of serving epidemiological research[15]; such data are within the domain of "infodemiology"[16]. Studies have shown that searches for diseases on the internet also reflect to some extent the epidemiology of these diseases. Google trends (available at https://trends.google.com) provides free access to search requests made to Google. The data are amassed *via* sampling algorithms proprietary to Google and are anonymized and categorized. Searches on the Internet may - according to some researchers - accurately reflect the epidemiology[16-18] of infectious[19-23], acute[24], or chronic[25] diseases, including, among others, coronary[24] or thyroid disease[19,25]. The use of Google trends, with the aim to study a wide range of medical topics, is becoming more widespread. In this work, we assessed the reported increased incidence of PP from the perspective of digital epidemiology, since the latter may be a useful adjunct to classical epidemiology.

MATERIALS AND METHODS

Data collection

We collected from Google trends (https://trends.google.com), the searches for 21 PP-related keywords/search terms in English internationally. We chose keywords/search terms in English because in previous studies we showed that with regards to Google trends, the volume of internet searches in English is vastly larger than in any other language (and practically dwarfs searches in other languages [18]). We limited the collection to the years 2017-2021 (Table 1). The years of the study were imposed by

Table 1 Worldwide searches in English for precocious puberty-related terms *vs* time

Search terms	Tau (*P* value)
Puberty-related terms *per se*	
"Puberty"	+0.351 (< 0.001)
"Precocious puberty"	+0.048 (0.250)
"Early puberty"	+0.130 (< 0.001)
Body-related terms	
"Hair"	+0.114 (0.008)
"Pubic"	-0.567 (< 0.001)
"Pubic hair"	-0.499 (< 0.001)
"Axillary"	+0.254 (< 0.001)
"Axillary hair"	+0.146, (< 0.001)
"Height"	+0.713 (< 0.001)
Female body-related terms	
"Breast"	-0.219 (< 0.001)
"Breast enlargement"	-0.497 (< 0.001)
Menstruation-related terms	
"Menarche"	+0.125 (0.003)
"Menses"	-0.556 (< 0.001)
"Period"	+0.584 (< 0.001)
Male body-related terms	
"Testes"	+0.119 (0.004)
"Testicles"	-0.506 (< 0.001)
"Testicular enlargement"	+0.024 (0.601)
"Penis"	-0.726 (< 0.001)
"Penis growth"	-0.226 (< 0.001)
"Scrotum"	-0.262 (< 0.001)
"Scrotum growth"	-0.006 (0.881)

Google Trends' algorithms: These were changed, according to Google, on January 1, 2017 and then on January 1, 2022. The PubMed database (https://pubmed.ncbi.nlm.nih.gov/) was searched, with no time limits. The search strategy, honed on the tentative effects of COVID-19 on PP, was as follows: "((precocious AND puberty) OR (early AND puberty)) AND COVID-19". There were 12 original articles (not case reports), all of which[5-8] reported a rise in PP cases[9-12] during the pandemic[13]. Additionally, we assessed Google trends local searches for selected terms, in English and local languages, in countries where a rise in PP has been reported in the medical literature (Table 2). Google does not report absolute internet search volumes. Instead, Google gives the sum of each search in the form of relative search volumes (RSVs)[26]. These RSVs are percentages relative to the peak search volume obtained during the specified time period and scaled by the total search volume for each specific search term. Consequently, these numbers do not represent absolute search volume numbers, but are normalized and presented on a scale from 0 to 100[26].

Data analyses

The collected searches (in RSV format) per each week were assessed vis-à-vis time (in weeks) and analyzed using Kendall's Tau test, with a statistical significance threshold of $P < 0.05$, using JASP v.0.16.3 (JASP Team, University of Amsterdam, Netherlands, 2022).

Table 2 Selected searches in English and local languages in countries where a rise in the reporting of precocious puberty has been reported *vs* time

Search terms	Tau (*P* value)
Italy (English & Italian)	
"Precocious Puberty"	NA
"Pubertà Precoce"	+0.046 (0.292)
"Early puberty"	NA
"Menarche"	NA
"Menarca"	+0.057 (0.169)
Turkey (English & Turkish)	
"Precocious Puberty"	NA
"Erken Ergenlik"	+0.050 (0.248)
"Early puberty"	NA
"Menarche"	NA
"Menarş"	+0.002 (0.996)
India (English, Marathi & Hindi)	
"Precocious Puberty"	+0.103 (0.014)
"Precocious Puberty (in Marathi)"	+0.118 (0.004)
"Precocious Puberty (in Hindi)"	NA
"Early puberty"	+0.052 (0.240)
"Menarche"	+0.371 (< 0.001)
"Menarche (in Marathi)"	NA
"Menarche (in Hindi)"	+0.041 (0.161)
Brazil (English & Portuguese)	
"Precocious Puberty"	NA
"Puberdade Precoce"	+0.362 (< 0.001)
"Early puberty"	NA
"Menarche"	+0.002 (0.959)
"Menarca"	+0.264 (< 0.001)
Spain (English & Spanish)	
"Precocious Puberty"	NA
"Pubertad precoz"	-0.007 (0.862)
"Early puberty"	NA
"Menarche"	-0.006 (0.904)
"Menarquia"	+0.038 (0.356)
South Korea (English & Korean)	
"Precocious Puberty"	-0.008 (0.427)
"Precocious Puberty (in Korean)"	NA
"Menarche"	0.012 (0.397)
"Menarche (in Korean)"	0.011 (0.395)

NA: Not available.

RESULTS

In the published reports of increased PP during COVID-19, in 2020, the reported cases were relatively disproportionally distributed, with 678 cases in Italy[2-6], 275 in Turkey[8-10], 237 in China[11], 22 in Brazil[12] and 155 in India[13]; Portugal was not included in further analyses (see below for details). In South Korea, with data obtained from the country's hospital records/healthcare database, 95524 children were diagnosed with PP in 2017 (9.89% boys), increasing over the years to 166645 children in 2021 (19.55% boys). These corresponded to 0.18% of South Koreans in 2017 to 0.32% of South Koreans in 2021[27].

Internationally, searches for three PP-related terms in English showed no noticeable change over the study period, while searches for eight terms showed a decrease. An increase was found over time in searches for nine PP-related terms (Table 1 and Figure 1). Of the 18 searches in English and local languages, in countries where a rise in PP has been reported, only 5 showed a significant increase over time (Table 2). In South Korea the reported total yearly numbers of PP cases has more than doubled over a timespan of 6 years; this increase is not mirrored by relevant internet searches. No searches were done for Portugal, since the only publication from this country, states - in a counterintuitive way - that there was an increase of PP cases at a single center, albeit with 598 cases in 2019 *vs* 471 cases in 2020[7]. Additionally, no searches were done for China (see the limitations section below). All the search data used are available online at: https://doi.org/10.5281/zenodo.7152041.

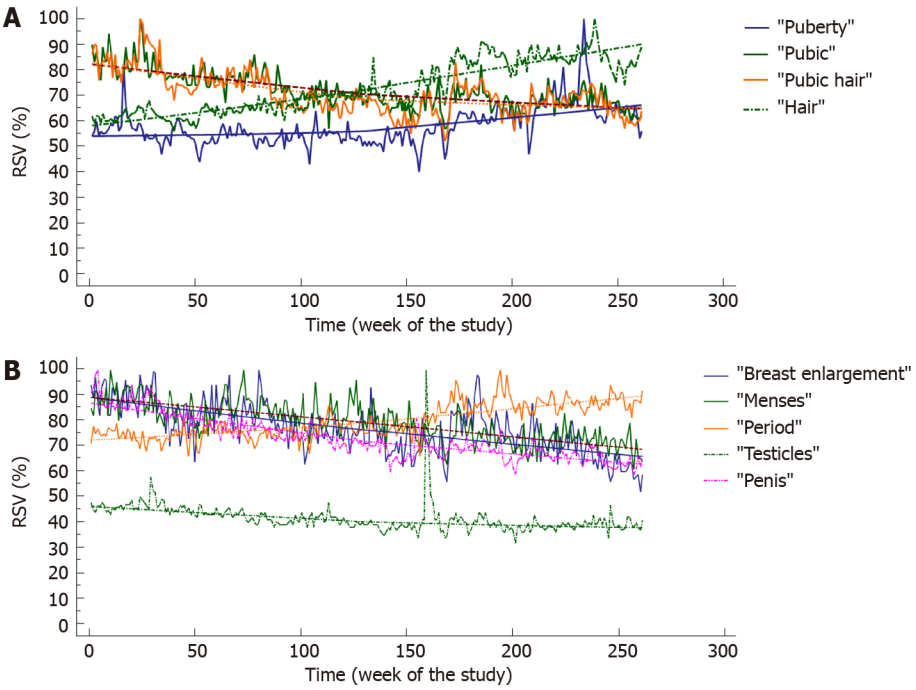
DISCUSSION

Over the study period more than half of the search terms showed little change or declined. Apparently, the most accurate and complete exposé of an increase in PP comes from South Korea. In this country, the reported total yearly numbers of PP cases has more than doubled over a timespan of 6 years[27]. Yet, this increase is not mirrored by relevant internet searches. The discrepancy between internet searches for PP and the reported increase in the literature is striking. The epidemiology of acute diseases (like influenza[19-23]) or chronic diseases (like thyroid[19,25] or cardiovascular ailments[24]) has been shown over the years to be reflected in associated and relevant internet searches, as they are gathered, summarized and reported in Google trends[16-18]. Lending credence to the association between disease cases and internet searches, in 2020-2021, the officially reported cases of COVID-19 worldwide were positively associated with relevant Google trends searches, as it has been shown in research works by our group as well by the work of other researchers[19,28,29]. In an analogous way, it would be expected that a true increase in the incidence of PP would also be aptly reflected in Google trends. If our findings are valid, then the literature is likely to show some bias and also to be distorted by the small - proportionally - number of reported PP cases.

For PP, its epidemiological profile is not clear worldwide, with vast differences across countries and continents, with some published reports in the medical literature presenting the incidence and others giving the prevalence of PP, adding to confusion[1]. The "gold standard" to help in the diagnosis/differential diagnosis of PP is the gonadptropin-releasing hormone test. It is laborious and costly. Tools using readily and easily available data may be handy in the diagnosis and eventual management of PP[30]. Additionally, there is a lack of validated instrument to assess the psychosocial impact of PP [31]. Internet-based tools could fill this gap.

The COVID-19 pandemic was an unprecedented event, with profound effects in social/psychological functioning[32], particularly during the lockdown periods; anxiety and interoception were affected[33]. Parents were very stressed during the early phases of the pandemic[34]. This could have also led to heightened vigilance and preoccupation with their children's welfare[35]. The provision of healthcare - at times - was greatly compromised. The referrals and diagnoses of PP under such circumstances may be prone to several biases[36,37]. The numbers of patients channeled to specialized centers may be prone to centripetal, diagnostic access and referral bias and the diagnoses at these centers may be prone to confirmation bias. Studies generated at these centers may be hampered by wrong size bias[38]. The known secular trend of decreasing age of puberty may also have played a role. Trends of increased PP incidence had been noted in Denmark[39] and Spain[40]. Regarding South Korea, the recent finding of increasing PP incidence is not novel[27]. A secular trend of increasing PP incidence had been also noted earlier, in the years 2004 to 2010 in the same country[41]. There are no clear causative factors for the reported rise in the incidence of PP. Excess sedentary lifestyle and rising obesity may play a role[42]. Thus, the epidemiology of PP is varied across different populations and countries; comparisons among them may not be straightforward.

There were limitations and caveats in our study. We collected only Google trends data, but as far as the internet is concerned, Google searches outnumber those of any other search engine, reaching an impressive percentage of 95% worldwide (https://gs.statcounter.com/search-engine-market-share; accessed October 30, 2022). An exception to this is China, where the internet is searched with local search engines, albeit without a feature analogous to that of Google trends. Moreover, we know that English-language searches outnumber searches in all other languages[18]. Another limitation was the



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Figure 1 Selected Internet searches worldwide (as relative search volumes) for non-sex-specific and sex-specific terms related to premature puberty. A: Non-sex-specific terms; B: Sex-specific terms. RSVs: Relative search volumes.

restrained choice of keywords in the searches; for the sake of homogeneity in the searches, the "related queries" feature of Google trends was not used. We did not evaluate for any periodicity because of the relatively short duration of pandemic. Medical research articles generate public interest when reported by news outlets[43]; Google searches are easily influenced by media items[43]. For the duration of the study no such interest was discernible, since the relevant medical articles on increased incidence of PP appeared in 2022, after the end of this study's time period.

CONCLUSION

The increase in the incidence of PP in the COVID-19 era, which is reported in the medical literature, is not fully reflected in internet searches. This is an evolving issue; hopefully further relevant studies will shed light on it.

ARTICLE HIGHLIGHTS

Research background

Recent publications from several countries have reported that more young people (mainly girls) are experiencing precocious puberty (PP)/menarche during the coronavirus disease 2019 (COVID-19) pandemic compared to the past. This variation is attributed to the stress of confinement, lack of exercise, obesity and disturbed sleep patterns. Studies have shown that searches for diseases on the internet also reflect to some extent the epidemiology of these diseases.

Research motivation

A common feature of the relevant papers on the rise of PP, however, is the relatively small number of reported cases. With this study we aimed to estimate, through internet searches for PP, any changes in the epidemiology of PP.

Research objectives

We assessed in Google trends searches for 21 PP-related terms in English internationally, in the years 2017-2021. Additionally, we assessed local searches for selected terms, in English and local languages, in countries where a rise in PP has been reported.

Research methods

Searches were collected in relative search volumes format and analyzed using Kendall's Tau test, with a statistical significance threshold of $P < 0.05$.

Research results

Internationally, searches for three PP-related terms showed no noticeable change over the study period, while searches for eight terms showed a decrease. An increase was found over time in searches for nine PP-related terms. Of the 17 searches in English and local languages, in countries where a rise in PP has been reported, 5 showed a significant increase over time.

Research conclusions

Over the study period, more than half of the search terms showed little change or declined. The discrepancy between internet searches for PP and the reported increase in the literature is striking. It would be expected that a true increase in the incidence of PP would also be aptly reflected in Google trends. If our findings are valid, then the literature may have been biased. The known secular trend of decreasing age of puberty may also have played a role.

Research perspectives

The increase in the incidence of PP in the COVID-19 era, which is reported in the medical literature, is not fully reflected in internet searches. This is an evolving issue; hopefully further relevant studies will shed light on it.

FOOTNOTES

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Country/Territory of origin: Greece

ORCID number: Athanasios Tselebis 0000-0002-2591-965X; Lina Zabuliene 0000-0002-7889-0862; Charalampos Milionis 0000-0003-2442-3772; Ioannis Ilias 0000-0001-5718-7441.

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ABOUT COVER

Peer Reviewer of *World Journal of Methodology*, Azzam A Magazachi, Professor, College of Medicine, Department of Clinical Sciences, University of Sharjah nd UAE University anked 1st, United Arab Emirates. amagazachi@sharjah.ac.ae

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MINIREVIEWS

Is mandible derived mesenchymal stromal cells superior in proliferation and regeneration to long bone-derived mesenchymal stromal cells?

Madhan Jeyaraman, Tushar Verma, Naveen Jeyaraman, Bishnu Prasad Patro, Arulkumar Nallakumarasamy, Manish Khanna

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Madhan Jeyaraman, Department of Orthopaedics, ACS Medical College and Hospital, Dr MGR Educational and Research Institute, Chennai 600056, Tamil Nadu, India

Madhan Jeyaraman, Department of Biotechnology, School of Engineering and Technology, Sharda University, Greater Noida 201310, Uttar Pradesh, India

Madhan Jeyaraman, Naveen Jeyaraman, Bishnu Prasad Patro, Arulkumar Nallakumarasamy, Manish Khanna, Department of Regenerative Medicine, Indian Stem Cell Study Group Association, Lucknow 226010, Uttar Pradesh, India

Tushar Verma, Naveen Jeyaraman, Arulkumar Nallakumarasamy, Department of Orthopaedic Rheumatology, Fellow in Indian Orthopaedic Rheumatology Association, Lucknow 226010, Uttar Pradesh, India

Naveen Jeyaraman, Department of Orthopaedics, Rathimed Speciality Hospital, Chennai 600040, Tamil Nadu, India

Bishnu Prasad Patro, Arulkumar Nallakumarasamy, Department of Orthopaedics, All India Institute of Medical Sciences, Bhubaneswar 751019, Odisha, India

Corresponding author: Madhan Jeyaraman, MS (Orth), FEIORA, FIRM, FROSM, FASM, PhD, Assistant Professor, Research Associate, Department of Orthopaedics, ACS Medical College and Hospital, Dr MGR Educational and Research Institute, Chennai 600056, Tamil Nadu, India. madhanjeyaraman@gmail.com

Abstract

Mesenchymal stromal cells (MSCs) are cells with the characteristic ability of self-renewal along with the ability to exhibit multilineage differentiation. Bone marrow (BM) is the first tissue in which MSCs were identified and BM-MSCs are most commonly used among various MSCs in clinical settings. MSCs can stimulate and promote osseous regeneration. Due to the difference in the development of long bones and craniofacial bones, the mandibular-derived MSCs (M-MSCs) have distinct differentiation characteristics as compared to that of long bones. Both mandibular and long bone-derived MSCs are positive for MSC-associated markers such as CD-73, -105, and -106, stage-specific embryonic antigen 4 and Octamer-4, and negative for hematopoietic markers such as CD-14,

-34, and -45. As the M-MSCs are derived from neural crest cells, they have embryogenic cells which promote bone repair and high osteogenic potential. *In vitro* and *in vivo* animal-based studies demonstrate a higher rate of proliferation and high osteogenic potential for M-MSCs as compared to long-bones MSCs, but *in vivo* studies in human subjects are lacking. The BM-MSCs have their advantages and limitations. M-MSCs may be utilized as an alternative source of MSCs which can be utilized for tissue engineering and promoting the regeneration of bone. M-MSCs may have potential advantages in the repair of craniofacial or orofacial defects. Considering the utility of M-MSCs in the field of orthopaedics, we have discussed various unresolved questions, which need to be explored for their better utility in clinical practice.

Key Words: Mandible; Long bone; Mesenchymal stromal cells; Osteogenic potential; Regeneration

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Core Tip: Due to the difference in the development of long bones and craniofacial bones, the mandibular-derived MSCs (M-MSCs) have distinct differentiation characteristics as compared to that of long bones. In vitro and *in vivo* animal-based studies demonstrate a higher rate of proliferation and high osteogenic potential for M-MSCs as compared to long-bones MSCs, but *in vivo* studies in human subjects are lacking. Considering the utility of M-MSCs in the field of orthopaedics, we have discussed various unresolved questions, which need to be explored for their better utility in clinical practice.

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INTRODUCTION

Mesenchymal stromal cells (MSCs) are cells with the ability to self-renew along with the ability to exhibit multilineage differentiation[1,2]. Initially, they were identified from the murine bone marrow (BM) as "plastic-adherent cells", which are mainly generated from the fibroblast colony-forming units (CFU-F). Friedenstein *et al*[3] first identified CFU-F by isolating adherent cells from the BM stroma of newborn rodents which can form discrete colonies. However, these cells are regulated by various mitogenic factors such as epidermal growth factor, platelet-derived growth factor, transforming growth factor-β, basic fibroblast growth factor, and insulin growth factor-1[4-6].

Previously, MSCs were given much attention due to their precious role in creating a supportive microenvironment in the hematopoietic tissue but later their precursor role was identified for the formation of skeletal tissue/bone[7,8]. MSCs in adults have been studied extensively in animals as well as humans and have been isolated from various tissues such as BM of long bones (including ilium, femur, tibia) and mandibular bone[9-11].

International Society for Cellular Therapy has suggested the identification criteria for mesenchymal progenitors i.e. these cells can express CD-73, -90, and -105 but cannot express CD-11b or -14, -19 or -79a, -34, -45, -34 and human leukocyte antigen (HLA) -DR[12,13]. MSCs are used in the treatment of non-healing ulcers or wounds, for promoting bone regeneration in cases with non-healing or delayed healing, and MSCs can differentiate into various tissue-specific cell types, which can promote angiogenesis. Treatment with these cells has shown promising results in wound healing by various mechanisms such as promoting re-epithelialization, improving granulation tissue, promoting angiogenesis, and reducing inflammatory reactions. MSCs are utilized in the management of chronic non-healing ulcers, diabetic ulcers, bed/pressure sores, and radiation-induced burns[14].

An electronic search was conducted until Dec 2022 including articles from January 2003 to December 2022 databases such as PubMed, Web of Science, Embase, and CNKI (China Knowledge Resource Integrated Database). The terms used for the search included: "mesenchymal stromal cell", "MSCs", "mandible", "long bone", "regenerative potential", "proliferation", and "regeneration". In this manuscript, we compared the proliferation and regenerative potential of mandible and long bones.

BONE MARROW-DERIVED MSCS

Bone marrow is the first tissue in which MSCs were identified and BM-MSCs are most commonly used in clinical settings. The Food and Drug Administration registered the first drug derived from BM-MSCs called "prochymal", a drug against Graft *vs* Host Disease[15]. MSCs derived from the BM have a unique ability to proliferate and differentiate into various cell types in the culture i.e. fibroblasts, chondrocytes, osteocytes, adipocytes, myogenic cells, *etc*. Apart from this, MSCs also can secrete potent bioactive cytokines, which help the MSCs to regulate other cell types[16-18]. MSCs can be obtained from BM of long bones which are appendicular bones derived from the mesoderm. However, maxillary and mandibular bones develop from the neural crest cells[19]. These differences in the development of the long bone and mandibular bones may reflect the difference in the properties of progenitor cells derived from different BM sites. Previous studies have reported phenotypic and functional differences in laboratory studies for cell proliferation, adipogenic potential, osteogenic potential, efficiency to form colonies, and cell surface markers[20-22]. These cells have therapeutic significance i.e. they can stimulate bone growth and promote the regeneration of the bone. MSCs have been suggested to be beneficial in the management of fractures with delayed union or non-union. These cells are documented to have certain advantages; first, these cells can migrate to the site of injury and promote regeneration; secondly, these cells suppress the local immune response; third, the quantity of the MSCs can be obtained in large amounts from patients themselves[23].

Overall, the efficacy of MSCs has been established *in vitro* studies. However, the survival of these cells *in vivo* largely depends upon depends on cell survival, osteogenic differentiation, and host cell recruitment. The major limiting factor affecting the therapeutic potential of MSCs is their low survival rates following transplantation. Literature suggests that transplanted MSCs cannot survive in the presence of temporal hypoxia or a harsh microenvironment where the MSCs of the donor are not able to survive and eventually undergo apoptosis[24]. The advantages of BM-MSCs include high stability in the culture, feasible accessibility to harvesting sites, and high osteogenic potential. The disadvantages of BM-MSCs include the painful BM harvesting process and the risk of infection by the procedure[25].

MANDIBLE DERIVED MSCS

The maxillofacial region is one of the richest sources of BM-MSCs. This region is comprised of bones particularly jaw bones, dental tissues, blood vessels, nerves, adipose tissue, and muscular tissue[11]. The MSCs from BM of the mandible (jaw) was first described in 2005 by Matsubara *et al*[20]. Neural crest cells [cranial, vagal, trunk, and cardiac] help in the development of the peripheral nervous system, orofacial and cranial bones including the mandible, melanocytes, smooth muscle cells, and endocrine cells[26,27]. The intramembranous ossification leads to the formation of craniofacial bones.

Features of M-MSCs

Due to the difference in the development of long bones and craniofacial bones, M-MSCs have distinct differentiation characteristics as compared to long bones[20,21]. Yamaza *et al*[28] studied the features of M-MSCs isolated from the mouse. They reported that M-MSCs are capable of forming adherent colonies due to the presence of a colony-forming unit (CFU) and the number of colonies was $55.3 \pm 9.07/1.5 \times 10^6$ cells/plate. The potential of doubling and rate of cell proliferation of M-MSCs are much higher than BM-MSCs. M-MSCs are positive for MSC-associated markers such as CD-73, -105, and -106, stage-specific embryonic antigen 4 (SSEA-4), and Octamer-4 (Oct-4) whereas it is negative for hematopoietic markers such as CD-14, -34, and -45. M-MSCs are weakly positive for c-Kit and strongly positive for Sca-1 (stem cell antigen-1).

In vitro evidence of superiority in lineages of M-MSCs

Lee *et al*[29] investigated the role of M-MSCs *in vitro* studies and observed the formation of mineral nodules as early as 14 d of the osteogenic differentiation, which tends to increase over time till 21 d. These cells can suppress T lymphocytes and thus have been recommended in acute graft *vs* host disease. Li *et al*[24] observed the growth of M-MSCs within 2 to 3 d of the culture and the proliferation time was also documented to be much earlier *in vitro* study. Cytometric analysis revealed strong expression of CD-29, -73, -90, and -105. M-MSCs have higher osteogenic and mineralization potential as compared to femoral BM-MSCs, but the serial passage *in vitro* reduces differentiation potentials[29]. Yamaza *et al*[28] observed M-MSCs from mice to have stronger suppressive effects on anti-CD3 antibody proliferation which activates T cells thereby suppressing T cell activation. M-MSCs produce NO in a higher amount as compared to BM-MSCs when stimulated with IFN-γ. The multilineage differentiation under osteogenic conditions revealed their differentiation into osteoblasts with increased activity of serum alkaline phosphatase (ALP) and increased mineralized nodule formation. Also, these cells exhibit higher expression of osteoblastic markers such as osteocalcin, RunX2, and ALP.

In vivo evidence of superiority in lineages of M-MSCs

Lee *et al*[29] reported a significantly higher rate of mineralization in the rat calvarial defects implanted with gel foam with M-MSCs as compared with gel foam only. The volume of new bone was 80.88% ± 0.68% for the gel foam with the M-MSCs group and only 49.87% ± 0.94% for only the gel foam group. Overall, M-MSCs have reported higher osteogenic potential with high site-specific bone regeneration capacity[20,21]. Various studies have documented the osteogenic potential of M-MSCs which helps in bone regeneration[30-32]. Deluiz *et al*[33] in their rat model study demonstrated that M-MSCs inoculation significantly promoted bone formation at 4 wk (22.75 ± 2.25 mm^3) as well as at 8 wk (64.95 ± 5.41 mm^3) as compared to acellular bone microparticles (2.34 ± 2.91 mm^3 and 42.73 ± 10.58 mm^3 at 4 wk and 8 wk respectively). The TRAP and osteocalcin-positive cells were also higher on immunohistochemical analysis at 4 wk in the cell-seeded group as compared to the acellular group. Yamaza *et al*[28] transplanted M-MSCs into immunocompromised mice along with a carrier [hydroxyapatite/tricalcium phosphate (HA/TCP)] and demonstrated increased osteogenic potential in the form of increased bone formation.

LONG BONE-DERIVED MSCS

MSCs were initially derived from the long appendicular bones and these bones are the principal source of MSCs in clinical settings owing to their feasible accessibility. The appendicular bones develop from mesoderm[34]. The most common location among the appendicular bone for isolation of MSCs is the iliac crest. The alternative sites include long bones (tibia, femur, humerus, radius) and sternum[34]. Literature suggests that MSCs properties as well as graft retaining properties of MSCs may vary depending upon harvesting sites[35].

Features of long bone-derived MSCs

As the sites of BM aspiration of appendicular bones are easily accessible, aspiration is easy[35]. These cells are positive for MSC-associated markers such as CD-29, -44, -73, -90, -105, -166, and HLA-ABC and negative for hematopoietic markers such as CD-14, -34, and -45[28,35]. The osteogenic potential of the MSCs helps in bone regeneration and bone repair. The MSCs have been utilized in the management of delayed union or non-union of fracture, osteogenesis imperfecta, osteoporosis, *etc.* Also, the MSCs can differentiate into chondrocytes, adipocytes, osteocytes, *etc*[36].

In vitro evidence in lineages of long bone-derived MSCs

Li *et al*[37] observed the appearance of colonies of femur-derived MSCs (F-MSCs) was scantly on the 2nd or 3rd day. Cytometric analysis revealed strong expression of CD-29, -73, -90, and -105. The cells derived from F-MSCs have osteogenic and mineralization potential, and the serial passage *in vitro* does not reduce the ability of differentiation of these cells. Proliferation is delayed but the cloning rate is higher. The osteogenic potential as evidenced by ALP lasted beyond 21 d. Lee *et al*[29] investigated the role of F-MSCs *in vitro* study and observed mineralization within 14 days these cells express CD-44, -72, -90, and -105, but failed to express CD-34 and -45.

In vivo evidence in lineages of long bone-derived MSCs

The F-MSCs have increased osteogenic potential when transplanted into immunocompromised mice as evidenced by the increased bone formation in a study by Yamaza *et al*[28]. Aghaloo *et al*[22] observed a primarily cartilaginous matrix following long bone-derived MSC implantation with good osteoblastic differentiation. The periosteum of long bones contains mesenchymal progenitors which have high proportions of EdU (DNA synthesis probe)-positive cells and possess the highest clonogenic ability. Apart from this, these progenitors have a lower rate of apoptosis with high proliferative properties[38]. A comparison of mandible *vs* long bone-derived MSCs is depicted in Table 1.

COMPARISON OF MSCS FROM FEMUR, TIBIA, HUMERUS, RADIUS, AND ILIUM

Recently, MSCs have been harvested from BM of long bones such as the femur (proximal and distal), tibia, humeral head, radius, ilium, *etc*[39,40]. The posterior part of the iliac crest is preferred for obtaining autologous stem cells as it contains the highest amount of nucleated cells (25.1–54.7) × 10^6 cells/mL, whereas the concentration of nucleated cells in the anterior iliac crest is (24.4–49) × 10^6 cells/mL. However, the mean number of nucleated cells in decreasing concentration has been reported from the proximal humerus (38.7 × 10^6 cells/mL), followed by the distal femur (25.9 × 10^6 cells/mL), humeral head, and proximal tibia (12.1 × 10^6 cells/mL)[39]. Mc Daniel *et al*[41] observed the highest BM aspirate, higher nucleated cells, and highest CFUs from the iliac crest. However, CFUs from bone marrow aspirate (BMA) of the iliac crest, femur, tibia, and humerus were 12692.3 ± 4981.4, 11235.2 ± 3451.6, 9433.9 ± 4065.1, and 9347.3 ± 3366.3 respectively whereas that from concentrated BMA aspirates, highest

Table 1 Comparison of mandible *vs* long bone-derived MSCs

Ref.	Features	Mandible derived MSCs	Long bone-derived MSCs
Lee *et al*[35], 2011	Aspiration time	10 min	2 min
Yamaza *et al* [28], 2011	No. of colonies	55.3 ± 9.07/ 1.5 × 10^6 cells/plate (Higher)	5.33 ± 0.58/ 1.5 × 10^6 cells/plate
Li *et al*[37], 2019		The appearance of colonies was early within 2-3 d of inoculation into the culture	The appearance of colonies of Femur- MSCs was scantly on the 2nd or 3rd day as compared to M-MSCs
Yamaza *et al* [28], 2011	Osteogenic potential	High	Low
Matsubara *et al*[20], 2005		High	Low
Aghaloo *et al* [22], 2010		higher activity of ALP and OCN expression suggesting higher osteogenic potential	Comparatively lower osteogenic potential
Li *et al*[37], 2019		After 21 d, M-MSCs showed loss of morphology, and dry staining was observed; *Runx2* gene expression was higher	After 21 d, F-MSCs showed obvious cell morphology
Yamaza *et al* [28], 2011	Doubling rate and cell proliferation	High	Low
Lee *et al*[29], 2019		Proliferation time (OD-0.82 ± 0.26) was also documented to be much earlier as compared to F-MSCs but doubling time was lower (22.6 ± 2.22 h)	Proliferation time was much delayed (OD-1.13 ± 0.41) as compared to M-MSCs but doubling time was earlier (35 ± 3.19 h)
Li *et al*[37], 2019		Proliferation time was also documented to be much earlier as compared to F-MSCs	Proliferation time was much delayed as compared to M-MSCs
Li *et al*[37], 2019	Arrangement of cells	On day 2, triangular, while after cell (tightly) fusion- these cells are arranged as paving stones	On day 2, elongated fibroblast-like morphology, while after cell (tightly) fusion- F-MSCs show vortex-like cloning center
Yamaza *et al* [28], 2011	Cell expression	Positive for MSC-associated markers such as CD-73, -105, and -106, SSEA-4, and Oct-4; Negative for hematopoietic markers such as CD-14, -34, and -45; Expresses SSEA-4 (6.4%) and Oct-4 (6%) in much higher proportion as compared to long bones	Positive for MSC-associated markers such as CD-73, -105, and -106, SSEA-4, and Oct-4; Negative for hematopoietic markers such as CD-14, -34, and -45. Expresses SSEA-4 (4.2%) and Oct-4 (2.6%) in lower proportion
Lee *et al*[35], 2011		Negative for hematopoietic stem cells such as for CD-14, -34, -45, and HLA-DR whereas positive for MSC markers such as CD-29, -44, -73, -90, -105, -166, and HLA-ABC	Negative for hematopoietic stem cells such as for CD-14, -34, -45, and HLA-DR whereas positive for MSC markers such as CD-29, -44, -73, -90, -105, -166, and HLA-ABC
Li *et al*[37], 2019		Strongly expressed CD-29, -73, -90, and -105 but negative for CD-31 and -34	Strongly expressed CD-29, -73, -90, and -105 but negative for CD-31 and -34
Aghaloo *et al* [22], 2010	Mineralization	Mandible BMSC were significantly larger and calcification was also more as compared to long bones; Tissue volume and bone volume was also larger	Less calcified as compared to M-MSCs
Lee *et al*[29], 2019		Mineralization appears within 14 d of osteogenic differentiation (mean-1.57 ± 0.05)	The mineral formation is higher (1.98 ± 0.05) as compared to M-MSCs at 14 d
Aghaloo *et al* [22], 2010	Histology	Characterized by increased and mature lamellar bone with marked osteoblastic rimming of bony trabeculae	The bone formed was primarily of the cartilaginous matrix with only peripheral bone formation

ALP: Alkaline phosphatase; BMSC: Bone mesenchymal stem cell; HLA: Human leukocyte antigen; MSCs: Mesenchymal stromal cells; M-MSCs: Mandibular-derived MSCs; F-MSCs: Femur-derived MSCs; OCN: Osteocalcin; SSEA-4: Stage-specific embryonic antigen 4; Oct-4: Octamer-4.

CFU was obtained from the iliac crest, followed by tibia, femur and least was from humerus.

LACUNAE IN UNDERSTANDING M-MSCS

Though M-MSCs has been utilized in animal studies and their osteogenic potential, immunomodulatory effect and clinical utility have been documented, studies in human are lacking and the mechanism depicting *in vivo* potential in therapeutic and clinical setting needs further elucidation. The factors affecting these cells when transplanted *in vivo* such as route of inoculation, time, indication for inoculation, and location of their inoculation need to be explored. Autologous M-MSCs potential is explored in previous studies, and literature elucidating the roles of allogenic M-MSCs in bone repair/ regeneration with risks of rejection needs further exploration. Despite the utility of M-MSCs in the field

of orthopaedics, there remain various unresolved questions, which need to be explored for their better utility in clinical practice.

AUTHOR'S OPINIONS

BM-MSCs have adherent properties that form the colonies and have osteogenic potential with the characteristic ability to differentiate into various types of cells such as osteoblasts, chondrocytes, adipocytes, *etc*. Irrespective of sites, BM-MSCs can suppress T lymphocytes and cell-mediated immunity supporting its utility in graft *vs* host disease. Concerning the accessibility and ease of obtaining the BM-MSCs, long bones are superior and the cells could be obtained as early as 2 min. However, the risk of infection is high[25] in the case where BM is derived from long bones. M-MSCs have a significantly higher number of CFUs, high proliferation rate, higher ALP activity, and high osteogenic potential as compared to MSCs derived from long bones, especially during the initial 14 d[28,41]. For the prolonged duration, the MSCs derived from BM-MSCs had higher activity and less apoptosis. The doubling time and cloning time are also superior for MSC derived from long bones as compared to M-MSCs. Therefore, we recommend the regenerative medicine researchers and experts to explore the regenerative potential of mandible derived MSCs in chondrogenesis and osteogenesis.

CONCLUSION

MSCs are of therapeutic significance for bone repair and regeneration. As M-MSCs are derived from neural crest cells, they have embryogenic cells which promote bone repair and have high osteogenic potential. *In vitro* and *in vivo* animal-based studies demonstrate a higher rate of proliferation and higher osteogenic potential for M-MSCs as compared to long-bones-derived MSCs, but *in vivo* studies including human subjects are still lacking. BM-MSCs have their advantages and limitations. M-MSCs may be utilized as an alternative source of MSCs which can be utilized for tissue engineering and promoting the regeneration of bone. M-MSCs may have potential advantages in the repair of craniofacial or orofacial defects.

FOOTNOTES

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Country/Territory of origin: India

ORCID number: Madhan Jeyaraman 0000-0002-9045-9493; Naveen Jeyaraman 0000-0002-4362-3326; Bishnu Prasad Patro 0000-0001-9497-9624; Arulkumar Nallakumarasamy 0000-0002-2445-2883; Manish Khanna 0000-0002-2890-869X.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Urinary tract injury during hysterectomy: Does surgeon specialty and surgical volume matter?

Emilee Khair, Fareeza Afzal, Sanjana Kulkarni, Beaux Duhe', Karen Hagglund, Muhammad Faisal Aslam

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Emilee Khair, Fareeza Afzal, Sanjana Kulkarni, Muhammad Faisal Aslam, Department of Obstetrics and Gynecology, Ascension St John, Detroit, MI 48236, United States

Beaux Duhe', Department of Obstetrics and Gynecology, St. George's University School of Medicine, Great River, NY 11739, United States

Karen Hagglund, Department of Medical Research, Ascension St John, Detroit, MI 48236, United States

Corresponding author: Emilee Khair, MD, Doctor, Department of Obstetrics and Gynecology, Ascension St John, 22101 Moross Rd, Detroit, MI 48236, United States.
emilee.khair@ascension.org

Abstract

BACKGROUND

Ureteral injury is a known complication of hysterectomies. Recent studies have attempted to correlate surgeon volume and experience with incidence of urinary tract injuries during hysterectomies. Some studies have reported that as surgeon volume increases, urinary tract injury rates decrease. To our knowledge, no studies have assessed the relationship between surgeon subspecialty and the rate of urinary tract injury rates during minimally invasive hysterectomy.

AIM

To determine the incidence of urinary tract injury between urogynecologists, gynecologic oncologists, and general gynecologists.

METHODS

The study took place from January 1, 2016 to December 1, 2021 at a large community hospital in Detroit, Michigan. We conducted a retrospective chart review of adult patients who underwent minimally invasive hysterectomy. After we identified eligible patients, the surgeon subspecialty was identified and the surgeon's volume per year was calculated. Patient demographics, medical history, physician-dictated operative reports, and all hospital visits postoperatively were reviewed.

RESULTS

Urologic injury occurred in four patients (2%) in the general gynecologist group, in one patient (1%) in the gynecologic oncologist group, and in one patient (1%) in

the urogynecologist group. When comparing high and low-volume surgeons, there was no statistically significant difference in urinary tract injury (1% *vs* 2%) or bowel injury (1% *vs* 0%). There were more complications in the low-volume group *vs* the high-volume group excluding urinary tract, bowel, or major vessel injury. High-volume surgeons had four (1%) patients with a complication and low-volume surgeons had 12 (4%) patients with a complication (P = 0.04).

CONCLUSION
Our study demonstrated that there was no difference in the urinary tract injury rate in general gynecologists *vs* subspecialists, however our study was underpowered.

Key Words: Minimally invasive hysterectomy; Urinary tract injury; Surgeon volume; High volume gynecologist; Low volume gynecologist

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Core Tip: Surgeon volume and experience have been shown to play a role in decreasing the number of urinary tract injuries during minimally invasive hysterectomies. One may conclude that since urogynecologists and gynecologic oncologists had additional training years after residency, they also have more experience. This may result in a decreased incidence of urinary tract injury during minimally invasive hysterectomies. To our knowledge, no studies to date have been done to assess this correlation.

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INTRODUCTION

Hysterectomy is a common gynecologic surgery in the United States. It is estimated that there are over three hundred thousand hysterectomies performed each year[1]. Ureteral injury is a known complication of hysterectomies, and it is estimated that between 52 and 82 percent of all iatrogenic urinary tract injuries occur during gynecology surgeries[2]. Studies have reported iatrogenic ureteral injury incidence as low as 0.18%[3] and as high as 2.2%[4]. These injuries increase the rates of patient morbidity and mortality such as sepsis and fistula formation[5].

The method of hysterectomy has been examined to assess this risk of urinary tract injury. Janssen *et al*[4] found that those undergoing abdominal hysterectomy had an increased risk of ureteral injury when compared with vaginal hysterectomy. Another study found that the incidence of urinary tract injury was lowest in laparoscopic supracervical hysterectomy (LSH), compared to laparoscopic assisted vaginal hysterectomy (LAVH) and total laparoscopic hysterectomy (TLH)[6].

More recently, surgeon volume and experience have been studied when assessing risk factors for urinary tract injury during hysterectomies. Vree *et al*[7] reported that high-volume surgeons (those performing greater than 51 hysterectomies per year) had shorter operative time and less estimated blood loss, but no difference in the rate of urinary tract injury when compared with low-volume surgeons (those performing less than 11 hysterectomies per year). However, another study demonstrated that patients who underwent benign hysterectomy by a high-volume surgeon (greater than 14.1 hysterectomies per year), were less likely to have bladder, ureteral, and intestinal injury when compared with those surgeons who performed less than 5.88 hysterectomies per year[8]. Janssen *et al*[4] reported that as surgeon experience increased, defined by a threshold of greater than 30 hysterectomies performed, the risk of ureter injury decreased from 2.2% to 0.5%. To our knowledge no studies have been performed evaluating the effect of surgeon subspecialty on urinary tract injury rates during minimally invasive hysterectomy.

MATERIALS AND METHODS

We conducted a retrospective chart review of adult patients who underwent minimally invasive hysterectomy (including laparoscopic and robotic methods) with and without concomitant procedures from January 1, 2016 to December 1, 2021. All procedures and postoperative care were done at a large urban hospital by a fellowship trained board-certified female pelvic medicine and reproductive surgery

(FPMRS) surgeon (also known as a urogynecologist), fellowship trained board eligible or board-certified gynecologic oncology surgeons, and board-certified general gynecologists. All patients who underwent the following surgeries with or without concomitant procedures were included: LSH, LAVH, TLH, and robotic hysterectomy. After we identified eligible patients, the surgeon subspecialty was identified and the surgeon's volume per year was calculated. Patient demographics, medical history, physician-dictated operative reports, and all hospital visits postoperatively were reviewed. Our primary outcome was the incidence of urinary tract injury between fellowship trained board-certified FPMRS surgeon, fellowship trained board eligible or board-certified gynecologic oncology surgeons, and board-certified or board eligible general gynecologists. Our secondary outcome was the incidence of urinary tract injury between high (defined by 30 or more minimally invasive hysterectomies per year) and low-volume surgeons (defined by less than 30 hysterectomies per year). To calculate a power analysis for our study, we used data reported by Mäkinen *et al*[9], who cited the rate of urinary tract injury as 4.4% and 1.3% for low- (less than 30 hysterectomies per year) and high-volume (equal to or greater than 30 hysterectomies per year) surgeons respectively. To show such an effect, with 80% power and alpha = 0.05, at least 452 patients were needed in each group, for a total of 904 patients. Descriptive statistics were generated to characterize the subjects. Continuous variables were described as the mean with standard deviation or median with 25th and 75th percentiles. Categorical variables were described as frequency distributions. Univariable analysis of factors associated with surgeon type and ureteral injury were assessed using Student's t-test, analysis of variance (ANOVA) followed by multiple pairwise comparisons using the Bonferroni correction of the *P* value, and the χ^2 analysis. Non-parametric tests were performed for data that were non-normally distributed, such as the Mann-Whitney U test and Kruskal-Wallis test. Analyses were conducted with SPSS version 25.0 and a *P* value less than 0.05 was considered to indicate statistical significance. All statistical analysis of this study were performed and/or reviewed by biomedical statisticians Karen Hagglund, MS and Susanna Szpunar, MPH, DrPH.

RESULTS

Primary outcome

In total 523 patients underwent minimally invasive hysterectomies performed during the study period. General gynecologists performed 255, the urogynecologist performed 196, and the gynecologic oncologists performed 102 procedures. Patient demographics are reported in Table 1. Patients in the general gynecologist group were younger than those in the urogynecologist and gynecologic oncologist groups. Patient race differed between groups. Patient history of cardiovascular disease differed between groups with those in the general gynecologist group having lesser incidence of cardiovascular disease ($P < 0.0001$). The average body mass index (BMI) also varied between groups with those in the urogynecologist (29.2 ± 6.3) having a lower BMI than those in the general gynecologist (32.6 ± 7.7) and gynecologic oncology (34.4 ± 9.2) groups ($P < 0.0001$).

Operating time and estimated blood loss also differed between groups. Across all time parameters (total set-up time, total operating time, and total room time), the urogynecologist had the longest times, followed by the gynecologic oncologist and then the general gynecologists ($P < 0.0001$). The urogynecologist [25.0 (20, 50)] had the least blood loss, while the general gynecologists [100 (50, 200)] had the most ($P < 0.0001$). These results can be found in Table 2. Length of stay did not differ between groups ($P = 0.93$) and can also be found in Table 2. Surgery type and concomitant procedures are detailed in Table 3. The urogynecologist performed more concomitant cystourethroscopies (100%) when compared to the general gynecologists (41%) and gynecologic oncologists (29%, $P < 0.0001$). The urogynecologist also performed more ureterolysis procedures (6%) than the general gynecologists (1%) and gynecologic oncologists (2%, $P = 0.01$). The general gynecologists performed less lysis of adhesions (22%) in comparison to the urogynecologist (35%) and gynecologic oncologist (34%, $P = 0.004$). Two percent of patients in both the general gynecologist and gynecologic oncologist groups underwent conversion to an open procedure. No procedures in the urogynecologist group underwent conversion to an open procedure.

Urologic injury occurred in four patients (2%) in the general gynecologist group, in one patient (1%) in the gynecologic oncologist group, and in one patient (1%) in the urogynecologist group. Bowel injury occurred in three (3%) of patients in the gynecologic oncologist group and there were none in the general gynecologist and urogynecologist groups. There were no cases of major vessel injury.

Secondary outcomes

A total of 42 surgeons performed minimally invasive hysterectomies at our institution during the specified time frame and were included in our study. Three of these surgeons performed 30 or more minimally invasive hysterectomies per year and qualified to be placed in the high-volume surgeon category. There were 280 patients in the high-volume group and 273 patients in the low-volume group. Patient demographics can be found in Table 4. Patient age and race differed between groups. Patient history of cardiovascular disease, hypertension, diabetes mellitus, and BMI also differed between groups. Total set up time, total operating time, and total room time all were significantly longer for

Table 1 Patient demographics-subspecialty, *n* (%)

	General gynecologist, *n* = 255; mean ± SD	Urogynecologist, *n* = 196; mean ± SD	Gynecologic oncologist, *n* = 102; mean ± SD	*P* value
Age (yr)	45.4 ± 8.3	58.9 ± 12.5	55.9 ± 11.3	< 0.0001
Race				0.001
Black	94 (37)	42 (21)	19 (18)	
White	146 (57)	139 (71)	73 (72)	
Other/Unknown	15 (6)	15 (8)	10 (10)	
BMI	32.6 ± 7.7	29.2 ± 6.3	34.4 ± 9.2	< 0.0001[a]
Cardiovascular disease	41 (16)	70 (36)	27 (27)	< 0.0001
Hypertension	83 (33)	86 (44)	48 (47)	0.01
Diabetes mellitus	25 (10)	24 (12)	18 (18)	0.12
Chronic lung disease	44 (17)	27 (14)	16 (16)	0.62
History of abdominal surgery	160 (63)	109 (56)	55 (54)	0.18

[a]Urogynecologist *vs* general gynecologist and gynecologic oncologist, $P < 0.0001$, general gynecologist *vs* gynecological oncologist, $P = 0.12$. BMI: Body mass index.

Table 2 Surgery characteristics and length of stay-subspecialty

	General gynecologist, *n* = 255; mean ± SD or median (25th %ile, 75th %ile)	Urogynecologist, *n* = 196; mean ± SD or median (25th %ile, 75th %ile)	Gynecologic oncologist, *n* = 102; mean ± SD median (25th %ile, 75th %ile)	*P* value
Total set-up time (minutes)	34.3 ± 8.2	51.1 ± 7.7	40.7 ± 9.8	< 0.0001[a]
Total operating time (minutes)	133.1 ± 57.8	257.8 ± 48.9	162.4 ± 69.2	< 0.0001[a]
Total room time (minutes)	192.1 ± 61.5	343.0 ± 51.9	231.1 ± 74.5	< 0.0001[a]
Uterine weight (grams)	181.2 ± 131.1	104.1 ± 72.7	150.9 ± 104.3	< 0.0001[b]
Estimated blood loss (mL)	100.0 (50, 200)	25.0 (20, 50)	50.0 (50, 100)	< 0.0001
Length of stay (d)	1.0 ± 0.7	1.1 ± 0.2	1.0 ± 0.5	0.93

[a]All comparisons, $P < 0.0001$.
[b]General gynecologist *vs* urogynecologist, $P < 0.0001$; general gynecologist *vs* gynecological oncologist, $P = 0.06$; urogynecologist *vs* gynecological oncologist, $P = 0.002$.

high-volume surgeons compared to low-volume surgeons. These comparisons can be found in Table 5. Uterine weight was higher in the low-volume surgeon group (179.0 0 ± 129.6) when compared to the high-volume surgeon group (117.50 ± 85.4, $P < 0.0001$). Low-volume surgeons also had an increased estimated blood loss when compared to high-volume surgeons [100.0 mL (50, 200) and 50.0 mL (20, 50) respectively, $P < 0.0001$]. The length of stay did not differ between groups. Patients in the high-volume group stayed 1.0 d ± 0.4 and those in the low-volume surgeon group stayed on average 1.0 d 0 ± 0.7 ($P = 0.98$).

High-volume surgeons performed mostly robotic hysterectomies (86%), while low-volume surgeons performed mostly LAVH (53%). While high-volume surgeons did perform ureterolysis more often than low-volume surgeons (5% *vs* 1%, $P = 0.01$), there was no significant difference in lysis of adhesions (31% *vs* 26%, $P = 0.17$). High-volume surgeons performed cystourethroscopy more often than low-volume surgeons (74% *vs* 44%, $P < 0.0001$). Two (1%) patients in the high-volume group were converted to open,

Table 3 Surgery type and concomitant procedures-subspecialty, *n* (%)

	General gynecologist, *n* = 255	Urogynecologist, *n* = 196	Gynecologic oncologist, *n* = 102	*P* value
Surgery				--
LAVH	144 (57)	0 (0)	25 (24)	
LSH	13 (5)	0 (0)	1 (1)	
TLH	47 (18)	0 (0)	13 (13)	
RATLH	50 (20)	196 (100)	63 (62)	
Concomitant procedures				--
None	19 (8)	0 (0)	1 (1)	
BS	168 (66)	13 (7)	13 (13)	
BSO	66 (26)	2 (10	88 (86)	
BS+SC	0 (0)	34 (17)	0 (0)	
BSO+SC	0 (0)	116 (59)	0 (0)	
SC	0 (0)	3 (2)	0 (0)	
BS+USLS	1 (0)	18 (9)	0 (0)	
BSO+USLS	1 (0)	7 (3)	0 (0)	
USLS	0 (0)	3 (2)	0 (0)	
Rectopexy	2 (1)	31 (16)	0 (0)	--
Cystourethroscopy	105 (41)	194 (99)	30 (29)	< 0.0001
Lysis of adhesions	55 (22)	68 (35)	34 (34)	0.004
Uterolysis	2 (1)	11 (6)	2 (2)	0.01
Conversion to open	5 (2)	0 (0)	2 (2)	--

BS: Bilateral salpingectomy; BSO: Bilateral salpingo-oophorectomy; SC: Sacrocolpopexy; LAVH: Laparoscopic assisted vaginal hysterectomy; LSH: Laparoscopic supracervical hysterectomy; RATLH: Robotic-assisted laparoscopic hysterectomy; TLH: Total laparoscopic hysterectomy; USLS: Uterosacral ligament suspension.

compared to five (2%) patients in the low-volume group were. When comparing high and low-volume surgeons, there was no statistically significant difference in urinary tract injury (1% *vs* 2%) or bowel injury (1% *vs* 0%). There were more complications in the low-volume group *vs* the high-volume group when looking at complications aside from urinary tract, bowel, or major vessel injury. High-volume surgeons had four (1%) patients with a complication and low-volume surgeons had 12 (4%) patients with a complication (P = 0.04). For high-volume surgeons, three patients had a postoperative wound infection or pelvic abscess, and one had a small bowel obstruction. For low-volume surgeons, four patients had vaginal cuff dehiscence, one patient had a small bowel obstruction, three patients required a blood transfusion postoperatively, one patient returned to the hospital with vaginal bleeding, and three patients had a postoperative wound infection or pelvic abscess.

DISCUSSION

We found no difference in the incidence of urinary tract injury when comparing subspecialists to general gynecologists or between high and low-volume surgeons. However, it is important to note that our study was underpowered, and therefore, a conclusion cannot be drawn. To our knowledge, this is the first study to look at differences in urinary tract injury rates in general gynecologists *vs* subspecialists. We plan to continue collecting data to gain a larger sample size to reach appropriate statistical power.

When comparing high and low-volume surgeons, low-volume surgeons had an increased rate of complications (excluding urinary tract injury and bowel injury) when compared to high-volume surgeons. This aligns with the findings of Rogo-Gupta *et al*[10], who reported that high-volume surgeons were less likely to have perioperative complications than low-volume surgeons. All high-volume surgeons in our study were subspecialists. As such, the increased incidence of complications

Table 4 Patient demographics of high *vs* low-volume surgeons, *n* (%)

	High-volume, *n* = 280, mean ± SD	Low-volume, *n* = 273, mean ± SD	*P* value
Age (yr)	58.1 ± 12.3	46.0 ± 8.8	< 0.0001
Race			
Black	60 (21)	93 (35)	0.002
White	197 (70)	161 (59)	
Other/Unknown	23 (8)	17 (6)	
BMI	30.9 ± 7.8	32.6 ± 7.8	0.01
Cardiovascular disease	92 (33)	46 (17)	< 0.0001
Hypertension	127 (45)	90 (33)	0.003
Diabetes mellitus	42 (15)	25 (9)	0.04
Chronic lung disease	41 (15)	46 (17)	0.49
History of abdominal surgery	153 (55)	171 (63)	0.06

BMI: Body mass index.

Table 5 Operating time of high *vs* low-volume surgeons

	High-volume, *n* = 280, mean ± SD	Low-volume, *n* = 273, mean ± SD	*P* value
Total set-up time (min)	47.5 ± 9.6	35.2 ± 9.3	< 0.0001
Total operating time (min)	224.4 ± 73.8	140.0 ± 62.7	< 0.0001
Total room time (min)	303.9 ± 82.1	200.3 ± 68.1	< 0.0001

seen in low-volume surgeons could be attributed to decreased surgical volume or lack of subspecialty training.

Limitations of this study include the inherent nature of a retrospective study and differences in surgical technique. This institution has only one urogynecologist and therefore these results cannot be generalized to results of all urogynecologists. There are also many physicians at this hospital that perform hysterectomies at multiple hospitals and, therefore, these procedures were not accounted for in this study. If the surgeries performed at other institutions were accounted for, there is a possibility that some of the generalists would qualify as high-volume surgeons.

Strengths of this study include a wide variety of general gynecologists and gynecologic oncologists to account for varied surgical technique and increased generalizability. All methods of minimally invasive hysterectomies are performed at this institution and therefore represented in this study. This study was also performed at a large institution in an urban city further increasing the generalizability. To our knowledge, this was the first study to look at differences in urinary tract injury rates in general gynecologists *vs* subspecialists. This study provides a guide for further and more widespread studies to be performed to investigate if a difference truly exists.

CONCLUSION

Surgeon volume has previously been shown to play a role in rate of urinary tract injury during minimally invasive hysterectomies. Although it has not been studied previously, it is reasonable to assume that this may also hold true for subspecialists *vs* general gynecologists, as subspecialists are usually high-volume surgeons. Our study demonstrated that there was no difference in the urinary tract injury rate in general gynecologists *vs* subspecialists, however our study was underpowered. We recommend a multicenter study to better analyze the potential differences.

ARTICLE HIGHLIGHTS

Research background

It is well known that urinary tract injury is a complication of hysterectomies. There have been many studies that aim to determine if surgeon volume has an impact on the incidence urinary tract injury during hysterectomies. However, no studies have compared subspecialists to general gynecologists when assessing the incidence of urinary tract injury.

Research motivation

Urinary tract injury increases morbidity for patients who undergo hysterectomy. Subspeciality training and surgeon volume are factors that should be assessed when determining the incidence of urinary tract injury in an effort to decrease patient morbidity.

Research objectives

Our primary outcome was the incidence of urinary tract injury between fellowship trained board-certified female pelvic medicine and reproductive surgery surgeon, fellowship trained board eligible or board-certified gynecologic oncology surgeons, and board-certified or board eligible general gynecologists. Our secondary outcome was the incidence of urinary tract injury between high (defined by 30 or more minimally invasive hysterectomies per year) and low-volume surgeons (defined by less than 30 hysterectomies per year).

Research methods

We conducted a retrospective chart review of adult patients who underwent minimally invasive hysterectomy. All patients who underwent the following surgeries with or without concomitant procedures were included: Laparoscopic supracervical hysterectomy, laparoscopic assisted vaginal hysterectomy, total laparoscopic hysterectomy, and robotic hysterectomy. After we identified eligible patients, the surgeon subspecialty was identified and the surgeon's volume per year was calculated. Univariable analysis of factors associated with surgeon type and ureteral injury were assessed using Student's *t*-test, ANOVA followed by multiple pairwise comparisons using the Bonferroni correction of the *P* value, and the χ^2 analysis. Non-parametric tests were performed for data that were non-normally distributed, such as the Mann-Whitney U test and Kruskal-Wallis test.

Research results

Urologic injury occurred in four patients (2%) in the general gynecologist group, in one patient (1%) in the gynecologic oncologist group, and in one patient (1%) in the urogynecologist group. Bowel injury occurred in three (3%) of patients in the gynecologic oncologist group and there were none in the general gynecologist and urogynecologist groups. There were no cases of major vessel injury.

Research conclusions

When comparing high and low-volume surgeons, there was no statistically significant difference in urinary tract injury (1% *vs* 2%) or bowel injury (1% *vs* 0%). There were more complications in the low-volume group *vs* the high-volume group when looking at complications aside from urinary tract, bowel, or major vessel injury.

Research perspectives

To our knowledge, this was the first study to look at differences in urinary tract injury rates in general gynecologists *vs* subspecialists. This study provides a guide for further and more widespread studies to be performed to investigate if a difference truly exists.

FOOTNOTES

Author contributions: Khair EL designed the study, collected data, and wrote and edited the manuscript; Afzal F, Kulkarni SP, and Duhe' BJ collected data for the manuscript; Hagglund K analyzed the data for the manuscript; Aslam MF edited the manuscript and assisted in study design; All authors have read and approved the final manuscript.

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Country/Territory of origin: United States

ORCID number: Emilee Khair 0000-0003-2811-1565; Fareeza Afzal 0000-0002-0158-8999; Sanjana Kulkarni 0000-0001-8481-3831; Beaux Duhe' 0000-0002-3222-9745; Karen Hagglund 0000-0003-2089-063X; Muhammad Faisal Aslam 0000-0002-4101-0382.

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LETTER TO THE EDITOR

Sexual function history taking in medicine

Raktim Swarnakar, Shiv Lal Yadav

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Raktim Swarnakar, Shiv Lal Yadav, Department of Physical Medicine and Rehabilitation, All India Institute of Medical Sciences, New Delhi, New Delhi 110029, India

Corresponding author: Raktim Swarnakar, MBBS, MD, Doctor, Department of Physical Medicine and Rehabilitation, All India Institute of Medical Sciences, New Delhi, New Delhi 110029, India. raktimswarnakar@hotmail.com

Abstract

Sexual history taking is important for the proper diagnosis and treatment of sexual dysfunction. It is often neglected in a clinical setting and it is also underreported by patients due to stigma and hesitation. Here we have described how we should take sexual function history taking during any sexual dysfunction.

Key Words: Sexual function; Sexual dysfunction; History taking; Medicine; Rehabilitation medicine

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Core Tip: Sexual history taking is crucial for the diagnosis and management of sexual dysfunction. It is often neglected in a clinical setting and it is also underreported by patients due to stigma and hesitation. Here we have highlighted how we should take sexual function history taking during any sexual dysfunction.

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TO THE EDITOR

Reproduction is a basic feature of living organisms for continuing their own species. Sexual function is vital for reproduction in this process[1]. Unfortunately, it is often neglected[2]. Especially sexual dysfunctions are often neglected as a medical condition [2]. It is also not thoroughly taught during the undergraduate medical curriculum and also during the postgraduate medical study[3]. Here we have highlighted how we should take sexual function history taking during any sexual dysfunction.

Sex

It refers to biological features that define as male or female, *etc*[4].

Sexual health

'Sexual health requires a positive and respectful approach to sexuality and sexual relationships, as well as the possibility of having pleasurable and safe sexual experiences, free of coercion, discrimination and violence. For sexual health to be attained and maintained, the sexual rights of all persons must be respected, protected and fulfilled'[4].

Sexuality

'A central aspect of being human throughout life encompasses sex, gender identities and roles, sexual orientation, eroticism, pleasure, intimacy and reproduction. Sexuality is experienced and expressed in thoughts, fantasies, desires, beliefs, attitudes, values, behaviours, practices, roles and relationships'[4].

Fertility

It is the capability to produce offspring through reproduction after sexual maturity. Infertility can be caused by a variety of conditions. Mishra *et al*[5] interestingly described that mild oxidative stress is beneficial but severe oxidative stress is harmful to male fertility. Hence any clinical condition leading to 'stress' must be addressed with priority in history taking.

Barriers

It is considered taboo in many areas in spite of its importance; no definite sex education exists especially in India or many countries; patient underreports his/her sexual problems to physicians due to stigma or taboo or hesitation; no specific guidelines for sexual history taking in the basic medical curriculum. Studies showed sexual history is taken as low as only 8% of cases at the clinical visit[6].

Overall comprehensive male and female sexual rehabilitation is taken care of under the Physical Medicine and Rehabilitation domain. Since rehabilitation medicine aims at the functional status improvement of patients, sexual function improvement is an important domain here. Furthermore, it is of utmost importance for primary care physicians as well.

STEPS OF SEXUAL HISTORY TAKING

There are multiple models are followed for sexual history taking: ALLOW (ask, legitimize, limitations, open discussion, work together), PLISSIT (permission, limited information, specific suggestions, intensive therapy) and BETTER (bring up, explanation, tell, time, educate, record) models[7-9]. (1) Make the patient comfortable before you go on asking private questions. Ask for permission or consent; (2) Initially use gender-neutral terms (spouse, better-half, partner *etc.* instead of girlfriend or boyfriend or husband-wife); (3) Then, ask in what gender patient wants to identify him/her *etc.* Is he/she comfortable with his/her gender? (4) Ask for the sexual orientation of the person, and decide whether the person is asexual, bisexual, heterosexual, or homosexual; (5) For males: Ask for psychogenic, reflexogenic erection. Ask for ejaculation (premature/delayed) and orgasm (absent, reduced/altered, normal), questions regarding scrotal hygiene/scrotal functioning/pain *etc*; (6) For females: Ask for psychogenic, reflexogenic genital arousal, genito-pelvic pain and menstruation. Also ask regarding pregnancy related history; (7) Check the quality of life by specific measurement scales [Emotional Quality of the Relationship Scale, Female Sexual Function Index, Sexual Attitude and Information Questionnaire, *etc.*]. Check how much it has been affected by sexual dysfunction; (8) Check the reason for dysfunction by history and examination; (9) Medical history to exclude medical causes of sexual dysfunction (cardiovascular disorder, diabetes, sexually transmitted disease, endocrine dysfunction, prostate dysfunction, spinal cord disorder/injury, brain injury/disorder *etc.*); (10) Fertility is an important domain that needs to be addressed in history, conditions that lead to 'stress' can influence fertility, especially in male[5]; (11) Medicine or substance abuse history: Antipsychotics, alcohol, recreational drugs *etc*; (12) Psychiatric disorders like depression/anxiety, post-traumatic stress disorder *etc*; (13) Relationship status with partner; and (14) Check 5 Ps (Partners, Practices, Protection from STIs, Past History of STIs and Pregnancy Intention)[10].

Thus, history should direct to identify the root cause so that further clinical examination and investigations can be proceeded.

FOOTNOTES

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Country/Territory of origin: India

ORCID number: Raktim Swarnakar 0000-0002-7221-2825.

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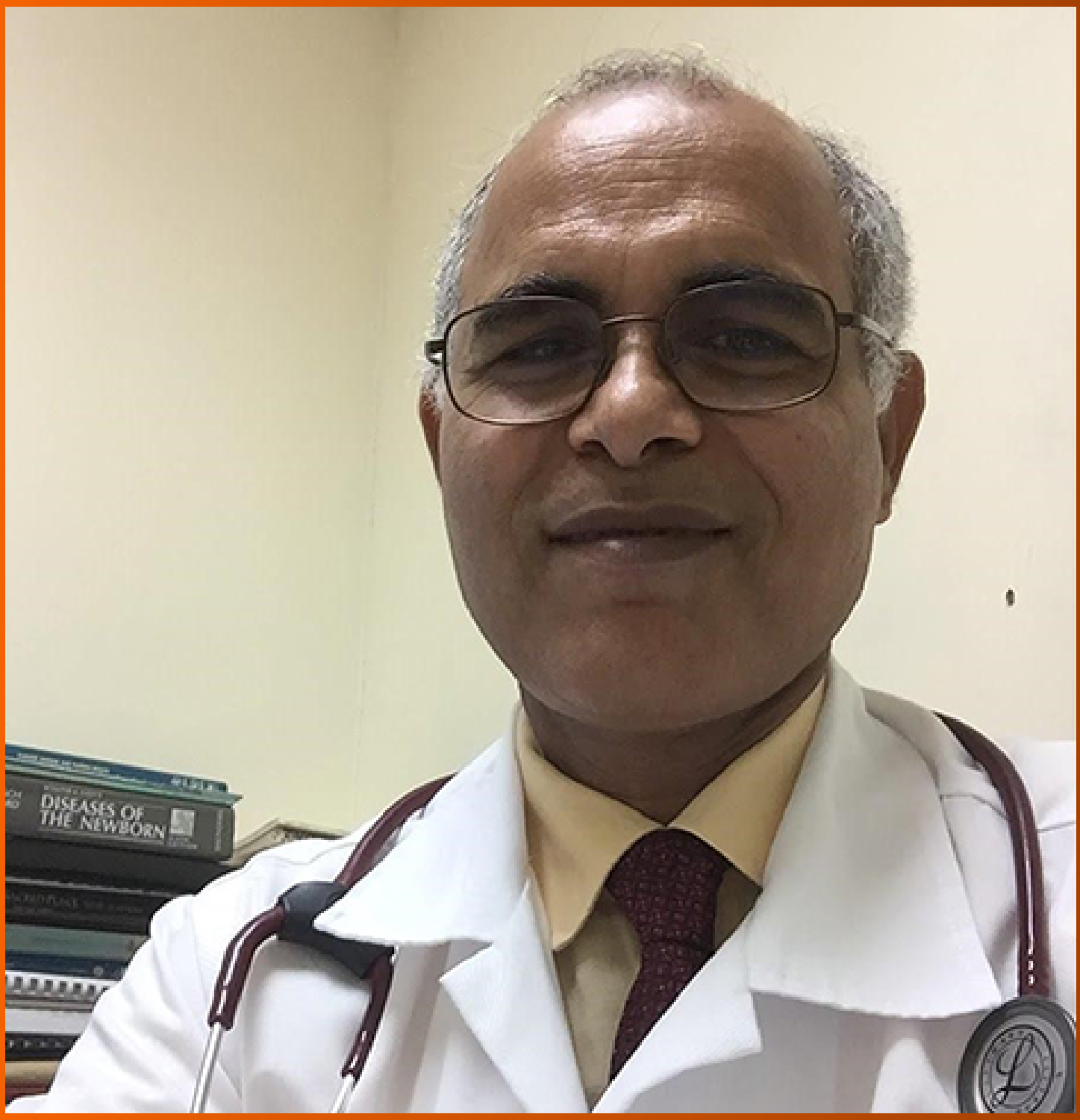


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ABOUT COVER

Peer Reviewer of *World Journal of Methodology*, Alok Kumar, Professor, MD, Coordinator, MBBS, Faculty of Medical Science, The University of the West Indies (Cave Hill), Barbados. alok.kumar@cavehill.uwi.edu

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REVIEW

Therapeutic potential of curcumin and its nanoformulations for treating oral cancer

Diptasree Mukherjee, Arunkumar Krishnan

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Diptasree Mukherjee, Department of Biochemistry, All India Institute of Medical Sciences, Bhubaneswar 751019, Odisha, India

Diptasree Mukherjee, Department of Medicine, Apex Institute of Medical Science, Kolkata 700075, West Bengal, India

Arunkumar Krishnan, Department of Medicine Section of Gastroenterology and Hepatology, West Virginia University School of Medicine, Morgantown, WV 26505, United States

Corresponding author: Arunkumar Krishnan, MBBS, Doctor, Department of Medicine Section of Gastroenterology and Hepatology, West Virginia University School of Medicine, PO Box 9161, 5th Floor HSC, Room 5500 Morgantown, WV 26505, United States.
dr.arunkumar.krishnan@gmail.com

Abstract

The global incidence of oral cancer has steadily increased in recent years and is associated with high morbidity and mortality. Oral cancer is the most common cancer in the head and neck region, and is predominantly of epithelial origin (*i.e.* squamous cell carcinoma). Oral cancer treatment modalities mainly include surgery with or without radiotherapy and chemotherapy. Though proven effective, chemotherapy has significant adverse effects with possibilities of tumor resistance to anticancer drugs and recurrence. Thus, there is an imperative need to identify suitable anticancer therapies that are highly precise with minimal side effects and to make oral cancer treatment effective and safer. Among the available adjuvant therapies is curcumin, a plant polyphenol isolated from the rhizome of the turmeric plant *Curcuma longa*. Curcumin has been demonstrated to have anti-infectious, antioxidant, anti-inflammatory, and anticarcinogenic properties. Curcumin has poor bioavailability, which has been overcome by its various analogues and nanoformulations, such as nanoparticles, liposome complexes, micelles, and phospholipid complexes. Studies have shown that the anticancer effects of curcumin are mediated by its action on multiple molecular targets, including activator protein 1, protein kinase B (Akt), nuclear factor κ-light-chain-enhancer of activated B cells, mitogen-activated protein kinase, epidermal growth factor receptor (EGFR) expression, and EGFR downstream signaling pathways. These targets play important roles in oral cancer pathogenesis, thereby making curcumin a promising adjuvant treatment modality. This review aims to summarize the different novel formulations of curcumin and their role in the treatment of oral cancer.

Key Words: Oral cancer; Oral squamous cell carcinoma; Analogues; Curcumin; Adjuvant therapy; Nanocurcumin; Curcumin nanoformulations; Curcumin analogues

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Core Tip: Oral cancer has a high disease burden worldwide. Oral squamous cell carcinoma is the most predominant subtype of oral cancer. The majority of oral cancers present at an advanced stage and are associated with a poor prognosis. Timely diagnosis and early treatment are critical to achieve a superior outcome. Surgery is the recommended treatment for oral cancer; other treatment modalities are radiotherapy with or without chemotherapy. Curcumin, a plant derivative, is one among the available adjuvant therapies that has been studied for its anticarcinogenic potential in the setting of various cancers. Curcumin has been proven to modulate intracellular signaling pathways that control cancer cell growth, inflammation, invasion, apoptosis, and cell death, with evidence supporting its use in cancer therapy. This review aims to summarize the molecular pathways involved in oral carcinoma pathogenesis, to explore different therapeutic interactions of curcumin, and to highlight the role of novel curcumin formulations in oral cancer treatment.

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INTRODUCTION

Oral cancer, a disease of predominant epithelial origin, is the most common subtype of cancer arising in the head and neck[1]. In 2020, more than 300000 new oral cancer cases were recorded globally[2]. Ninety percent of oral cancer cases are histologically diagnosed as oral squamous cell carcinomas (OSCCs)[3]. Oral cancer is widely prevalent in developing countries of South Central Asia (*e.g.*, India, Sri Lanka, Pakistan) and Melanesia, with a lesser disease burden in developed countries[4]. Oral cancer is the leading cause of death due to cancer in the Indian male population[5]. High incidence rates of oral cancer have been linked to alcohol consumption, tobacco smoking, betel nut chewing, and human papillomavirus (HPV) infection[6]. Despite diagnostic and therapeutic advances, the global 5-year survival rate remains less than 50%[7].

The primary treatment of oral cancer is based on the cancer stage. Surgery is the mainstay of multimodal therapy, which also includes radiotherapy and systemic treatment (chemotherapy and/or targeted agents)[8]. Chemotherapy has proven to increase treatment efficacy and improve overall survival, but has significant adverse effects and the potential for development of drug resistance[9]. As such, combination of these therapies with an adjuvant treatment to bolster their efficacy is urgently needed.

Among adjuvant therapies is curcumin, a phytochemical isolated from the turmeric plant *Curcuma longa*. Curcumin has been reported in a plethora of studies to have anti-infectious, antioxidant, anti-inflammatory, hepatoprotective, cardioprotective, thrombo-suppressive, anti-arthritic, chemopreventive, and anticarcinogenic properties[10]. Studies have shown the therapeutic role of curcumin in various cancers, including oral cancer[11]. Curcumin acts on numerous molecular targets, including signal transducer and activator of transcription 3 (STAT3), activator protein 1 (AP-1), protein kinase B (PKB also known as Akt), Notch 1, nuclear factor κ-light chain enhancer of activated B cells (NF-κB), Wnt, mitogen-activated protein kinase (MAPK), epidermal growth factor receptor (EGFR), and respective downstream signaling pathways, which are known to play key roles in oral cancer pathogenesis[10,12].

The hydrophobic nature of curcumin leads to poor bioavailability, and the sensitivity of soluble curcumin in physiological pH has limited its use in clinical practice[13]. However, nanotechnology-based techniques have made possible various novel formulations of curcumin such as liposomes, nanoparticles, micelles, phospholipid complexes, and analogues, to improve its tissue-level absorption and increase its pharmacological efficacy[14]. Studies have shown favorable results with the use of nano-formulated curcumin in the setting of epithelial cancers. Thus, it follows that curcumin may have a therapeutic role in oral cancer treatment as an adjuvant[15-17]. The present review aims to summarize the key properties of curcumin and its novel formulations, along with their role in oral cancer treatment.

ORAL CANCER

Oral cancer comprises neoplasms affecting any region of the oral cavity. The oral cavity is divided into distinct anatomic subsites, including lip, oral tongue, floor of the mouth, buccal mucosa, upper and lower gingiva, retromolar trigone, and hard palate[18]. However, 90% of oral cancers are histologically diagnosed as OSCCs[3]. OSCCs commonly present as nonhealing ulcers or growths. Early in the disease, lesions can appear as flat, discolored areas (*i.e.* erythroplakia or leukoplakia)[19]. Invasion of surrounding tissues can present with neck masses, trismus, referred ear pain, or specific sensory changes[20]. In cancer of the lip, there is often an exophytic, crusted lesion invading the underlying muscle with tissue damage in the adjacent lip[21]. Oral cancers are often diagnosed late due to an asymptomatic phase with fast progression and early metastasis[22]. Furthermore, the staging of oral cancer plays a significant role in survival rate, with early-stage (I and II) and advanced-stage (III and IV) lesions having a 5-year survival rate of 80% and 50% or less, respectively[22].

There are multiple pathways involved in oral carcinogenesis leading to genetic mutation (*e.g.*, H-ras, K-ras), gene deletions (*e.g.*, loss of chromosome 9p21 or 3p), promoter methylation (*e.g.*, p16, Ras association domain family member 1), amplification of oncogenes and oncoproteins (*e.g.*, EGFR, myc, bcl-2, ras, raf, stat-3, or cyclin D1) and inactivation of tumor suppressor genes (*e.g.*, p53)[23].

PROPERTIES OF CURCUMIN

Curcumin is a yellow spice derived from the roots (rhizomes) of *Curcuma longa*, commonly known as turmeric[24]. Turmeric contains curcuminoids, comprising curcumin, demethoxy curcumin (DMC), and bis-demethoxycurcumin (BDMC)[25]. In 1910, the principal ingredient of curcumin was identified by Gupta *et al*[26] as diferuloylmethane. Curcumin is known as 1, 7-bis (4-hydroxy-3-methoxy phenyl)-1, 6-heptadiene- 3, 5-dione (1E-6E) by International Union of Pure and Applied Chemistry nomenclature. It has a molecular weight of 368.4 g/mol and a melting temperature of 183 °C[27].

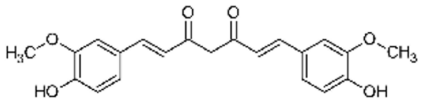
Curcumin contains 2 aromatic ring systems with o-methoxy phenolic groups linked with α- and β-unsaturated β-diketone moiety (Figure 1)[28]. The absorption bands of curcumin exist in the visible spectrum (410 nm-430 nm) and the ultraviolet spectrum (250 nm–270 nm)[14]. At 488 nm, curcumin is excited by lower fluorescent yield emission in the 500 nm-530 nm range, which can be detected by flow cytometry and confocal microscopy[29]. Curcumin is insoluble in water and readily soluble in polar solvents with keto-enol tautomerism[30]. The keto-form predominates in acid or neutral solutions, with the enol-form being predominant in alkaline solutions[31].

The bioavailability of curcumin is around 1% according to various animal studies, suggesting a requirement of high doses of curcumin (3600 mg to 12000 mg) to achieve beneficial effects[32]. It is known that curcumin's solubility in water (0.0004 mg/mL at pH 7.3) is poor, giving rise to challenges with oral administration[33]. One study has shown that curcumin has no toxic effect in patients with colorectal cancer when its oral dose is at least 3600 mg[34]. Curcumin undergoes rapid metabolism in the liver and gets excreted in the feces[35]. Curcumin is transformed into dihydrocurcumin and tetrahydrocurcumin (THC) and consequently converted into glucuronide conjugates[36,37]. In intestinal mucosa, kidney, and liver, the conjugative enzyme activity for glucuronidation and sulfation of curcumin has been discovered[37,38]. Another study demonstrated that a considerable portion of orally administered curcumin was conjugated to glucuronide in the intestine; later, the conjugated compound entered the portal vein and underwent additional conjugation to form glucuronide/sulfate metabolites of curcumin in the liver[38].

Curcumin, having a vast range of effects on various human diseases, plays an anti-tumorigenic role in different cancers by affecting multiple pathways of cancer progression[10-12]. In addition, it has been shown to have different effects on normal cells *vs* cancer cells, including a higher uptake by cancer cells [39]. The anticancer effects of curcumin are predominantly mediated through its regulation of various transcription factors, growth factors, inflammatory cytokines, protein kinases, and other oncogenic molecules, as summarized in Figure 2[10,11,33]. Curcumin metabolites (THC, hexahydrocurcumin, and octahydrocurcumin) also have anticancer properties[40,41]. However, the major factor restricting the use of curcumin as a novel chemotherapeutic agent is reduced bioavailability which is attributed to its poor absorption, rapid degradation, fast metabolism, and systemic elimination. Notably, as an anti-cancer drug, curcumin should be administered in a sufficiently high concentration; however, at these concentrations, patients have shown intolerance to bulk doses of the substance[33]. Using an advanced delivery system to increase the bioavailability of curcumin with satisfactory parenteral administration is the most promising solution to these challenges.

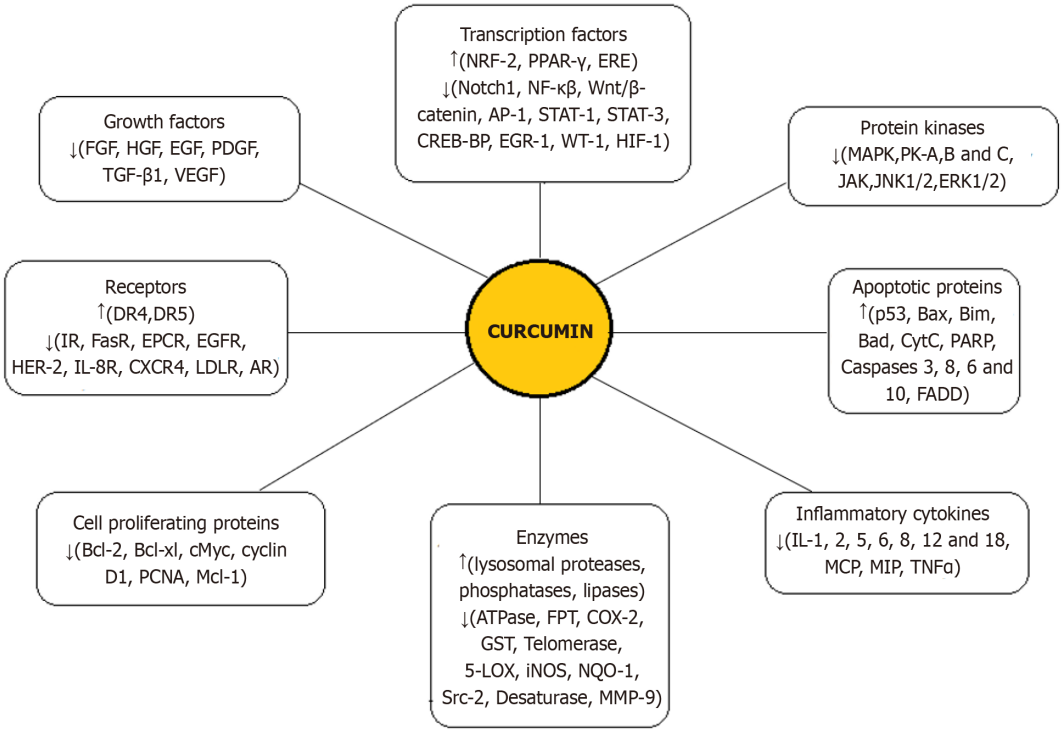
EFFECTS OF CURCUMIN ON ORAL CANCER

Curcumin is a potent agent that inhibits cell growth and deoxyribonucleic acid (DNA) synthesis in oral cancer cells[41]. Treatment with curcumin promotes the cell cycle's G(2)/M phase arrest, accompanied



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Figure 1 Chemical structure of curcumin.



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Figure 2 Effects of curcumin on multiple molecular targets involved in carcinogenesis. ↑: Upregulated; ↓: Down regulated. AP-1: Activator protein 1; AR: Androgen receptor; CREB BP: CREB Binding protein; CXCR: Chemokine receptor; DR4, DR5: Death receptor 4, 5; EGF: Epithelial growth factor; EGFR: Epithelial growth factor; EPCR: Endothelial cell protein C receptor; ERE: Estrogen response element; FADD: FAS-associated death domain; Fas R: Fas receptor; FGF: Fibroblast growth factor; FPT: Farnesyl protein transferase; GST: Glutathione S transferase; HGF: Hepatocyte growth factor; HIF 1: Hypoxia inducible factor 1; iNOS: Inducible Nitric oxide synthase; IR: Insulin receptor; JNK: Jun N-terminal kinase; LDLR: Low density lipoprotein receptor; MAPK: Mitogen activated protein kinase; MCP: Monocyte chemo-attractant protein; MIP: Macrophage inflammatory protein; MMP: Matrix metalloproteinase; NQO-1: NAD(P)H dehydrogenase (quinone) 1; NRF 2: Nuclear factor erythroid 2-related factor 2; PARP: Poly ADP- Ribose polymerase; PCNA: Proliferating cell nuclear antigen; PDGF: Platelet derived growth factor; PK: Protein kinase; PPAR γ: Peroxisome proliferator-associated receptor γ; STAT: Signal transducer and activator of transcription; TGF β 1: Transforming growth factor β 1; TNF-α: Tumor necrosis factor α; VEGF: Vascular endothelial growth factor.

by a decrease in cyclin B/cyclin-dependent kinase 1 and cell division cycle 25C protein levels. It induces apoptosis of oral cancer cells *via* reduction of Bcl-2 levels, reduction in mitochondrial membrane potential, promotion of the active forms of caspase-3, and the release of apoptosis-inducing factor (AIF) and endonuclease G from mitochondria[42]. Curcumin and curcuminoids like DMC and BDMC have shown autophagic and apoptotic activity[43,44].

Studies have shown that curcumin significantly inhibits the carcinogen-activating enzyme cytochrome P450 family 1 subfamily A member 1, which mediates benzo(a)pyrene diol bioactivation in both OSCC cells and oral mucosa[45]. Arecoline exposure is another significant risk factor for the development of oral cancer, and treatment with curcumin markedly inhibits arecoline-induced Snail expression[46]. One study showed that administration of curcumin at 100 mg/kg for 12 wk in a rat model with 4-nitroquinolone-1-oxide (4-NQO)-induced oral cancer markedly decreased the expression of proliferating cell nuclear antigen, anti-apoptosis markers (*e.g.*, Bcl-2), suppressors of cytokine signaling 3 and 1, and STAT3. It also minimized cellular atypia and reduced expression of vimentin, E-cadherin, N-cadherin, and TWIST1, which represent epithelial-mesenchymal transition (EMT) events [47]. Combining local and systemic C3 complex (a purified mixture of curcumin, BDMC, and DMC) effectively targets cancer cell proliferation. This combination inhibits 4NQO-induced tumorigenesis *via* modulation of fibroblast growth factor-2/fibroblast growth factor receptor-2[48]. Moreover, curcumin inhibits the activation and expression of host transcription factors AP-1 and NF-κB, which bind to a cis-regulatory region of the HPV genome. This effect is concentration- and time-dependent, leading to the

suppression of HPV16/E6 transcription and the subsequent prevention of oral carcinogenesis[49]. Curcumin triggers the activation of p38, which then interacts with binding elements in insulin-like growth factor binding protein-5, leading to the activation of the transcription factor CCAAT/enhancer binding protein α (C/EBPα). This also results in the suppression of oral carcinogenesis[50].

Additionally, curcumin reduces oral cancer cell viability and invasion by downregulating Notch 1 and NF-κB[12]. It also induces G2/M phase cell cycle arrest in a dose-dependent fashion by inhibiting the phosphorylation of EGFR and its downstream signaling molecules Akt, extracellular signal-regulated kinase (ERK1/2), and STAT3[12]. Treatment of oral cancer cells with curcumin, BDMC, and DMC leads to the production of reactive oxygen species (ROS), activation of caspase-8, -9, and -3, a decrease in the levels of matrix metalloproteinases (MMP), the release of AIF, and an alteration in the expressions of EGFR, PI3K, p-AKT, NF-κB, AMP responsive protein kinase, and MAPK[44]. In an *in vivo* OSCC model, curcumin has also been observed to suppress the expression of cyclo-oxygenase-2[51].

The oncogenic microRNA miR-31 is upregulated in OSCC, and curcumin downregulates the expression of this molecule in OSCC, leading to an attenuation of AKT activation and downregulation of C/EBPβ[52]. Moreover, curcumin inhibits oral cancer cell proliferation by upregulating miR-9 expression in a dose-dependent manner and suppressing Wnt/β-catenin signaling[53]. Furthermore, curcumin can also enhance the antitumor immune response by inhibiting the expression of programmed cell death ligand 1 and pSTAT3 leading to an increase in CD8+ T-cells and a decrease in T regulatory cells and myeloid-derived suppressor cells[54]. Cancer-associated fibroblasts (CAFs) are activated fibroblasts in the tumor microenvironment that play a critical role in cancer development[55]. Curcumin can reverse the phenotype of CAFs to that of peri-tumor fibroblast-like cells by downregulating the expression of α-smooth muscle actin (a unique marker for CAFs) and inhibiting the secretion of pro-carcinogenic cytokines such as transforming growth factor-β1, MMP2, and stromal cell-derived factor-1[56]. This results in decreased cancer invasion, as evidenced by a reduced release of EMT mediators in treated CAFs and reversal of EMT in treated tumor cells[57].

Hepatocyte growth factor (HGF) signaling plays an important role in EMT induction and contributes to cancer cell invasion and metastasis[58]. Curcumin inhibits HGF-induced EMT and cell motility in oral cancer cells, acting on HGF receptor c-Met and blocking the downstream activation of the pro-survival ERK pathway[59]. It also decreases proliferation in cell lines with mesenchymal characteristics and causes cell death with a dose-dependent decrease in cell–cell adhesion[60]. Curcumin treatment has been found to suppress MMP-2, MMP-9, and MMP-10, which are linked to cancer cell migration and invasion in oral cancer[61,62].

Studies have shown that curcumin can enhance the efficacy of standard platinum-based chemotherapy for treating oral cancer, resulting in significant tumor growth suppression in cell lines and mouse xenografts[63,64]. These results highlight the potential of using subtherapeutic doses of cisplatin in combination with curcumin to effectively suppress tumor growth and minimize cisplatin's toxic side effects.

Furthermore, curcumin has a radio-sensitizer effect in OSSC and exhibits synergistic antiproliferative activity when combined with cetuximab (an anti-EGFR monoclonal antibody) in cisplatin-resistant oral cancer cells[65,66]. A study of combinations of curcumin and metformin demonstrated a reduction in tumor volume and improvement of overall survival of experimental animals, as evidenced by downregulation of cancer stem cell markers CD44 and CD133[67]. Another study showed that olaparib (a poly-ADP ribose polymerase inhibitor), when combined with curcumin *in vitro* and *in vivo* (mouse model), causes DNA damage, inhibits cell proliferation and topoisomerase activity, reduces the expression of base excision repair components, induces apoptosis, and decreases tumor volume[68].

CLINICAL TRIALS USING CURCUMIN FOR ORAL LESIONS

Multiple clinical trials are ongoing or have been completed investigating the efficacy of curcumin against human diseases including oral pathologies. Kuriakose *et al*[69] conducted a study on oral leukoplakia, a potentially malignant oral cavity lesion with no effective treatment available. In this study, subjects with oral leukoplakia underwent a randomized, double-blinded, placebo-controlled phase IIB clinical trial with curcumin. Clinical and histological response assessments showed a significantly better outcome with curcumin treatment. Notably, the therapy was well-tolerated, and a significant and long-lasting clinical response was observed after treatment with curcumin at a dose of 3.6 g administered over 6 mo[69]. Furthermore, recent studies have shown that topical curcumin effectively treats oral mucositis[70]. Currently, a phase II randomized trial (double-blind, placebo-controlled) is ongoing to assess the therapeutic effects of curcumin in patients with stage III-IV head and neck cancer and cancer-associated anorexia-cachexia[71].

NOVEL FORMULATIONS OF CURCUMIN IN ORAL CANCER TREATMENT

Curcumin analogues

Several investigations have attempted to improve curcumin's therapeutic effectiveness and pharmacokinetic profile by developing new analogues[72,73]. The synthetic curcumin analogues that have been studied include the EF series (EF24, EF31, and UBS109), the FLLL series (FLLL11, FLLL12, FLLL31, FLLL32, and FLLL62), the GO-Y series, the 4-arylidene curcumin analogues AC17, B19 [(1E,4E)-1,5-bis(2,3-dimethoxy phenyl) penta-1,4-dien-3-one], CDF (difluorinated curcumin), and 4-[3,5-bis(2-chlorobenzylidene-4-oxo-piperidine-1-yl)-4-oxo-2-butenoic acid] CLEFMA, the diarylidenylpiperidones series, DM-1 (sodium 4-[5-(4-hydroxy-3-methoxyphenyl)-3-oxo-penta-1,4-dienyl]-2- methoxy phenolate), and dimethoxycurcumin[74]. In addition, some of these new analogues have been reported to have more potent anticancer properties than curcumin, and may have more beneficial antioxidant, antimalarial, and anti-inflammatory properties than the parent compound[75-79].

A few analogues including EF24, CDF, and FLLL12, exhibit enhanced physicochemical properties such as improved solubility and bioavailability, allowing them to overcome the limitations of curcumin [80,81]. In addition, some analogues have shown to increase the efficacy of chemotherapeutic agents and to overcome issues of resistance when combined therapy is used[82,83]. These analogues have shown promising results in breast, prostate, colon, and head-neck squamous cell cancers[77,84-87]. In a study of oral cancer cells, Chuprajob *et al*[88] found that curcumin analogues with the 1,4,6-trien-3-one function are more potent than the curcuminoid types. Also, structural variations in the analogues enhanced their potency; for example, the meta-oxygen function of the aromatic ring is more potent than those in the ortho and para positions, and the free phenolic hydroxy group is more potent than in the corresponding methyl analogues[88]. Furthermore, some analogues showed fewer toxic effects than curcumin when applied to normal cells[88]. In 2017, Lin *et al*[89] found that EF24 exhibited antitumor activity on CAL-27 oral cancer cells by deactivating the MAPK/ERK signaling pathway.

Several curcumin analogues have been developed in recent years, and most of the analogues have shown mechanisms of action similar to that of curcumin. However, some have unique mechanisms that are not associated with curcumin. For instance, the B19 analogue inhibits thioredoxin reductase 1, leading to ROS-mediated endoplasmic reticulum stress, whereas the AC17 analogue blocks proteasome function by inhibiting the deubiquitinase activity of 19S regulatory particles; neither of these mechanisms are seen with curcumin[90,91]. Further studies are required to evaluate the specific benefits of these inhibition pathways in cancer treatment.

Despite their promising potential, key parameters for the clinical development of many promising analogues remain unknown, and further attention should be given to the study of their pharmacokinetics. To reduce drug-associated toxicities and improve bioavailability, targeted drug delivery through alternative formulation has been gaining attention. Some studies have shown that curcumin analogues can be conjugated to homing moieties to direct their delivery and accumulation at specific sites. Certain homing moieties have been tested, including hyaluronic acid (HA)-targeted nanomicelles, HA dendrimers, folic acid-conjugated CDF, and EF24 conjugated to coagulation factor VIIa to target tissue factor[92-95]. These potential agents have yet to undergo clinical trials and cost-effective production strategies. Further *in vivo* studies can pave the way for clinical trials and future applications.

Nanoformulations of curcumin

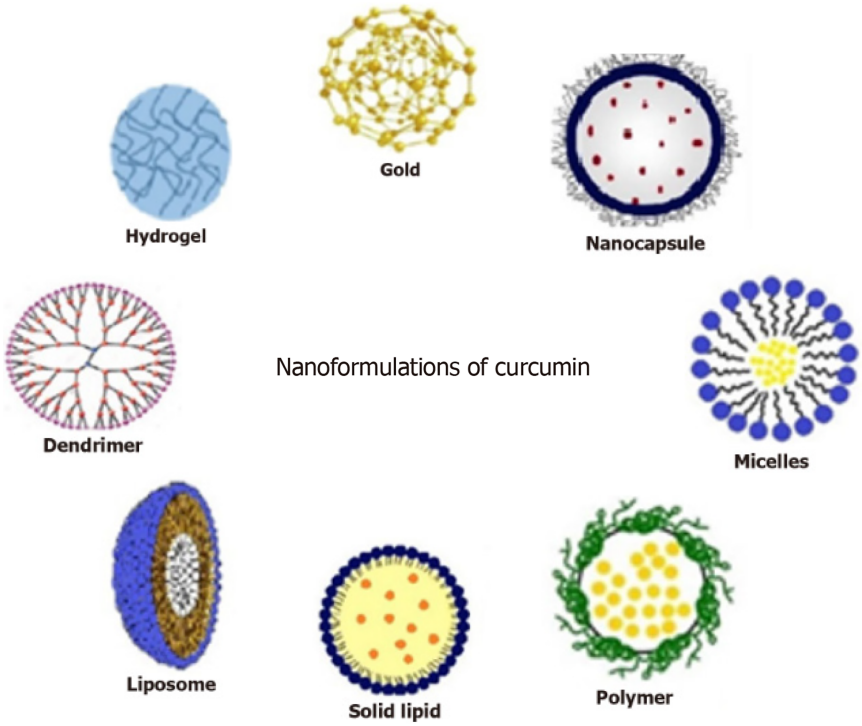
Nanotechnology has led the way in developing nanoscale drug delivery systems. Hydrophobic molecules such as curcumin can benefit from improved bioavailability as a result of the surface, small size, quantum size, and quantum tunnel effects of nanoparticles[96,97]. Several novel strategies have been developed to design curcumin nanoparticles as targeted drug-delivery systems, and these have been studied in various disease states, including cancer[33] (Figure 3).

The following is a summary of the different nanotechnology-based drug delivery modalities for potential use with curcumin.

Liposomes: These are spherical, closed phospholipid vesicles that incorporate drugs in the inner aqueous layer and have been widely used to enhance the bioavailability and efficacy of curcumin. In recent years, several liposomal curcumins with polymeric conjugates have been modified to achieve better clinical outcomes[98]. Nanoliposomes have shown properties such as sustained drug release, enhanced tumor targeting, minimized toxicity to healthy cells, and a lower dose[99].

Polymer micelles: These represent an excellent drug delivery system for curcumin, as they can overcome issues with poor solubility, low stability, and poor bioavailability. Encapsulating curcumin within cationic micelles like cetyltrimethylammonium bromide or dodecyl trimethyl ammonium bromide can enhance drug loading capacity, increase water solubility, reduce toxicity, and limit degradation[100]. Nanomicelle curcumin has been shown to prevent and treat oral mucositis caused by head and neck radiotherapy and chemotherapy[101,102].

Polymer nanoparticles: Polymer nanoparticles are another effective drug delivery system, owing to their high biocompatibility and ease of circulation in the bloodstream for longer periods. Synthetic



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Figure 3 Nanoformulations of curcumin used in various studies.

polymer conjugates such as chitosan, d,l-lactide-co-glycolide (PLGA), polyethylene glycol (PEG), poly (n-butyl) cyanoacrylate, silk fibroin, N-isopropyl acrylamide, and hydrophobically modified starch are commonly used. Curcumin-loaded nanoparticles using PEG-5000 as a carrier stabilizer for PLGAs have shown results in mouse models of cancer, with higher cellular uptake and induction of apoptosis both *in vitro* and *in vivo*[103].

Solid lipid nanoparticles: Solid lipid nanoparticles consist of natural lipids (*e.g.*, lecithins or triglycerides) that remain solid at 37 °C. These molecules protect labile compounds from chemical degradation and improve bioavailability. Curcumin-loaded solid lipid nanoparticles have shown enhanced cellular uptake and are promising anticancer agents against breast cancer cells *in vitro*[104].

Inclusion complexes: Inclusion complexes are formed of cyclodextrins and cyclic oligosaccharides composed of 6 to 8 glycosyl monomeric units (α-1,4 linked). They are widely used to improve stability, enhance water solubility, increase bioavailability, and reduce bitterness. β-Cyclodextrin is commonly used to form an inclusion complex with curcumin by solvent evaporation or pH shift techniques[105].

Solid dispersions: Solid dispersions have improved curcumin's physicochemical and pharmacokinetic activities. Wet-melting and subsequent freeze-drying are common strategies for preparing crystal and amorphous forms in which curcumin is dispersed in an inert carrier at a solid-state[106].

Magnetic nanoparticles: Drug-loaded magnetic nanoparticles can be directed to cancer-affected tissues under external magnetic fields to offer a targeted drug delivery option. Entrapping curcumin in an Fe3O4-curcumin conjugate with oleic acid or chitosan in the outer shell results in the formation of nano-sized, fluorescent-magnetic, water-dispersible nanoparticles with increased cellular uptake and enhanced bioavailability[107].

Microspheres and microcapsules: This approach encapsulates drugs or molecules like curcumin within polymeric particles to improve efficacy and organ-targeted bioavailability. Particles that have been used include camptothecin, rutin, zedoary oil, andrographolide, and eudragit S-100[108].

Emulsions: Emulsions refer to small-droplet dispersions comprising of oil and water mixtures that are stabilized by surfactant molecules to form interfacial films. These represent a lipid-based drug delivery system possessing numerous advantages, including thermodynamic stability, improved drug dissolution, and increased solubility[109]. High-speed and high-pressure homogenization procedures using triacylglycerol and Tween-20 as emulsifiers produce tiny microemulsion droplets. Incorporating curcuminoids into nanoemulsions has been shown to increase oral bioavailability[110].

Nanogels: Nanogels are 3-dimensional polymer networks with high drug loading capacity, high dispersion stability, targeted drug delivery efficiency, fast drug releasing properties, and increased drug delivery across cellular barriers. Moreover, they are easy to modify chemically. Curcumin has been used as a nanogel for targeted therapy[111].

Nanoparticle curcumin: Nanoparticle curcumin is a pure form of curcumin that is processed into nanoparticles of approximately 200 nm in size without carrier conjugates. *In vitro* and *in vivo* studies have shown that these nanoparticles of curcumin exhibit increased cellular uptake and enhanced anticancer effects due to their size, surface charge, and surface area[112-114].

Niosomes: Niosome nanocapsules are drug carriers composed of non-ionic surfactants that form a bilayered structure with hydrophobic and hydrophilic parts in an aqueous medium. Niosomes offer numerous advantages, including improved pharmacokinetics, drug stability, therapeutic effects, and reduced side effects of the administered drug[115].

Using nanoformulation-based combination therapy has gained popularity as a potent drug delivery system, often overcoming the limitations of conventional therapeutic agents. This delivery system has shown to improve intracellular drug concentrations and enhance the synergistic activity for cancer therapy[116-118]. Specific curcumin novel formulations have been studied for their efficacy in treating oral cancer, with encouraging findings (Table 1)[119,120].

In 2012, Lin *et al*[121] conducted a study to assess the effects of curcumin microemulsion on oral cancer cell lines. They found that exposure to curcumin-containing microemulsions for a brief period produced cytotoxic effects in the cancer cells. However, adding ultrasound enhanced these effects in OSCC-25 cells[121]. This observation is likely attributable to enhanced curcumin delivery to the cell by the fusion of microemulsion droplets with cell membranes or by overcoming transport limitations *via* ultrasound-induced mixing and/or heating. These ingestible microemulsions can be therapeutic in concentration-adjusted doses and have tissue-targeting properties when combined with ultrasound. Studies have shown that curcumin nanoparticles (Cur-NPs) possess significantly greater bioavailability and water solubility than free curcumin[122,123]. In a 2013 study by Chang *et al*[103] investigating CAL27-cisplatin-resistant human oral cancer cells (CAR cells), water-soluble PLGA Cur-NPs enhanced the drug effect. Cur-NPs increased ROS production, upregulated the expression levels of cleaved caspase-3/caspase-9, cytochrome c, apoptotic protease activating factor-1, AIF, and Bax, and downregulated the expression of Bcl-2. Cur-NPs also triggered the intrinsic apoptotic pathway by regulating the function of multiple drug resistance proteins 1 (MDR1) and the production of ROS in CAR cells[103]. Previous studies have also reported that MDR1 (a cell surface permeability glycoprotein) is a significant target of Cur-NPs[124,125]. In this study, treatment with Cur-NPs decreased MDR1 mRNA and protein levels in CAR cells, indicating the induction of CAR cell apoptosis, representing a potential treatment for cisplatin-resistant oral cancer.

Curcumin is phototoxic in the presence of oxygen[126,127]. Singh *et al*[128] demonstrated the use of organically-modified silica nanoparticles (SiNps) as a vehicle for the delivery of curcumin in human oral cancer cells. The results showed improved uptake of curcumin and phototoxicity in cancer cells. Incubation time-dependent cytotoxicity, inhibition of NF-κB activity, suppression of NF-κB-regulated proteins involved in invasion (MMP-9), angiogenesis (*via* vascular endothelial growth factor), and inflammation (tumor necrosis factor α) were observed with curcumin-SiNp. These results suggest that the curcumin–SiNp formulation has significantly improved anti-cancer effects over free curcumin in the dark and upon exposure to light[128]. These findings are likely the result of increased oxidative stress induced in the cancer cells upon visible light exposure in the presence of oxygen. The curcumin–SiNp formulation also enhances the stability of curcumin at physiological pH and increases its aqueous solubility.

In 2015, Mazzarino *et al*[129] conducted a study on the effect of mucoadhesive polycaprolactone (PCL) nanoparticles coated with chitosan and loaded with curcumin as a treatment for oral cancer. This study used the nanoprecipitation method to prepare the chitosan-coated PCL nanoparticles with curcumin loading[130]. The nanoparticles showed mucoadhesive properties, as evidenced by interaction with the glycoprotein mucin through electrostatic forces. *In vitro* studies showed that these novel curcumin nanoparticles significantly decreased the viability of SCC-9 human oral cancer cells by inducing apoptosis[129]. The study also suggested that drug retention in the mucosa after treatment with chitosan-coated curcumin-loaded nanoparticles could be helpful for local therapy in numerous diseases.

Gold nanorods (GNRs) are known for their photothermal activity and inherent tumor-targeting properties[131]. In 2018, Zhu *et al*[132] developed a novel system for combined plasmonic photothermal therapy and chemotherapy using the tumor microenvironment and near-infrared responsive gold nanorod-drug conjugates (Au NR@Curcumin). This study tested the antitumor effects of Au NR@Curcumin on human lung, liver, and oral carcinoma cells and found that it showed more potent cytotoxicity than the free drug. Additionally, oral cancer cells demonstrated cell cycle S phase arrest. The study suggested that Au NR@Curcumin could be effective at inducing instant photothermal killing of the cancer cells, even at a low irradiation power density[132]. In 2020, Ghosh *et al*[133] developed a multimodal nanoconjugate by functionalizing the GNR surface with a cytotoxic nucleoside [5-fluoro-2′-

Table 1 Oral cancer studies with novel curcumin formulations

Curcumin formulations	Study type	Results	Ref.
Liposomes	*In vitro*	Size of vesicle attributed to enhanced release of curcumin and cytotoxicity in the SCC9 cells	Gosangari *et al*[119], 2012
Cur microemulsion	*In vitro*	Damaged and ruptured OSCC 25 cells, cell death enhanced by ultrasound	Lin *et al*[121], 2012
PLGA Cur- NP	*In vitro*	Increased ROS production, upregulated caspase-3/caspase-9, cytochrome c, Apaf- 1, AIF, Bax, downregulated Bcl-2	Chang *et al*[103], 2013
Cur-SiNP	*In vitro*	Cytotoxicity by inhibition of NF-κB activity, suppression of MMP-9, angiogenesis (VEGF), and inflammation (TNF-α) in the dark as well as on exposure to light	Singh *et al*[128], 2014
Trienone analogues of curcuminoids	*In vitro*	1,4,6-trien-3-one analogue has more potent cytotoxicity than the curcuminoid type function in oral cancer cells	Chuprajob *et al*[88], 2014
Cur-loaded chitosan-coated PCL nanoparticle	*In vitro*	Mucoadhesive properties decreased SCC9 cell viability by inducing apoptosis	Mazzarino *et al*[129], 2015
Cur analogue EF24	*In vitro*	Anticancer activity on CAL-27 cancer cells *via* deactivation of the MAPK/ERK signaling pathway	Lin *et al*[89], 2017
Gold nanorod-drug conjugates (Au NR@Curcumin)	*In vitro*	Cancer cell cycle S phase arrest, the photothermal killing of the cancer cells	Zhu *et al*[132], 2018
NP Cur	*In vitro*	Chemoprotective nature of Cur towards 5-FU induced cell toxicity, antioxidant effect, altered expression of apoptotic proteins Bcl2 and Bax	Srivastava *et al*[112], 2018
	In vitro	Chemo-adjuvant property of NP Cur with Cetuximab	Mukherjee *et al*[136], 2022
	In vitro	Cytotoxicity *via* apoptosis, luminescence property of NP Cur acting as a theranostic agent	Essawy *et al*[135], 2022
PGA- Gef/Cur NP	*In vitro*	NPs internalized into SAS cells, decreased cell viability, and induced apoptotic cell death via caspase-3,9 and mitochondria-dependent pathway	Lai *et al*[134], 2019
	In vivo	Suppressed tumor size compared to the free Gef/Cur-treated group	
Mucoadhesive nanostructured Cur	*In vitro, Ex vivo*	Improved cytotoxicity, enhanced Cur release, and permeation while selectively targeting cancer cells	Ferreira *et al*[137], 2019
DNA Cur complex	*In vitro*	Enhanced cellular delivery of Cur increased cancer cell cytotoxicity in combination with FdU nucleotides	Ghosh *et al*[133], 2020
Nano micelle Cur	*In vitro*	Improved controlled-release of Cur, enhanced cellular uptake, apoptotic cell death by changing the mitochondrial membrane potential	Kumbar *et al*[120], 2022
Cur-loaded noisome	*In vitro*	Significant cytotoxicity compared to free curcumin after 24 h	Fazli *et al*[138], 2022
	In vivo	Injection use (systemic) was shown to be more effective than the use of mouthwash (topical)	

AIF: Apoptosis-inducing factor; Apaf-1: Programmed cell death ligand 1; CAR cells: Cisplatin-resistant human oral cancer cells; Cur: Curcumin; FdU: 5-fluoro-2'-deoxyuridine; 5-FU: 5-fluorouracil; MAPK: Mitogen-activated protein kinase; MDR 1: Multiple drug resistance proteins 1; MMP-9: Matrix metalloproteinase 9; Nano-CU: Nanoparticle of curcumin; NF-κB: Nuclear factor κ-light-chain-enhancer of activated B cells; NP: Nanoparticle; OSCC: Oral squamous cell carcinoma; PCL: Polycaprolactone; PGA- Gef/Cur NP: γ-polyglutamic acid-coated Gefitinib and curcumin-loaded nanoparticles; PLGA: D,l-lactide-co-glycolide; ROS: Reactive oxygen species; SCC: Squamous cell carcinoma; SiNp: Silica nanoparticle; TNF-α: Tumor necrosis factor α; VEGF: Vascular endothelial growth factor.

deoxyuridine (FdU)]-containing DNA hairpin followed by hydrophobic complexation of curcumin. This study showed that curcumin could be noncovalently complexed into small DNA hairpins for enhanced cellular delivery. This system caused increased cytotoxicity in SCC 131 oral cancer cells when administered in combination with FdU nucleotides, demonstrating its potential for advanced cancer therapy[133].

Several studies have investigated the potential of curcumin nanoparticles in combating oral cancer. Srivastava *et al*[112] found that Nano-CU, a curcumin nanoparticle, exhibited chemoprotective

properties against 5-fluorouracil-induced toxicity in oral cancer cells. Nano-CU was found to have an antioxidant effect, and altered the expression of apoptotic proteins Bcl-2 and Bax in treated cells[112]. Another study by Lai *et al*[134] explained the anticancer properties of gefitinib (Gef) and curcumin-loaded NPs in human oral cancer SAS cells *in vitro* and SAS cell xenografted tumors *in vivo*. The results indicated that γ-polyglutamic acid-coated (PGA)-Gef/Cur NPs could be internalized into SAS cells and significantly decrease the total cell viability. Both free Gef/Cur and γ-PGA-Gef/Cur NPs induced apoptotic cell death *via* caspase-3, caspase-9, and mitochondria-dependent pathways. *In vivo* studies showed that γ-PGA-Gef/Cur NPs significantly suppressed tumor size compared to the free Gef/Cur-treated group[134]. In 2022, Essawy *et al*[135] developed nanoparticle curcumin using a more straightforward and cost-effective solvent-antisolvent precipitation technique and studied its effect on oral cancer cells. This study found promising cytotoxic results *via* apoptosis in contrast to the necrotic effect observed using doxorubicin in the cell lines. The authors also reported the observed luminescence of the nanoparticle curcumin, qualifying it as a double theranostic agent[135]. In another study, co-treating oral cancer cells with nanoparticle curcumin (approximately 200 nm size) and cetuximab showed higher cytotoxicity than cetuximab alone[136]. The above mentioned studies highlight the potential chemo-adjuvant role of curcumin nanoparticles in combating oral cancer.

In 2019, Ferreira *et al*[137] aimed to develop nanostructured gel formulations containing curcumin for oral cancer therapy. The authors showed that the use of this novel curcumin led to rapid incorporation and localization in the hydrophobic portion of nanometer-sized polymeric micelles, resulting in increased retention after application in the oral cavity. Cytotoxicity testing showed that the formulation selectively targeted cancer cells moreso than healthy cells. Therefore, these systems may improve the physicochemical characteristics of curcumin by increasing its release and permeation and enhancing its cancer cell-targeting properties[137]. Recently in 2022, Fazli *et al*[138] found that curcumin-loaded niosomes significantly inhibited the growth and necrosis of oral cancer cells compared to free curcumin. Histopathological specimens from rats with induced oral cancer showed that niosome curcumin treatment effectively inhibited cancer growth. The authors also highlighted that the injectable curcumin-loaded niosome (*i.e.* for systemic use) was more effective than the mouthwash form of application (*i.e.* for topical use)[138]. In light of these promising findings, future studies should be designed to explore the outcomes of novel curcumin formulations in preclinical and clinical trials.

CHALLENGES AND FUTURE DIRECTIONS

Oral cancer is a highly malignant disease with a poor 5-year survival rate and limited treatment options, underscoring the importance of adjuvant therapy. Curcumin, known for its pleiotropic effects and potential therapeutic benefits, has shown promise as a treatment choice for patients with cancer. This molecule has shown improvement in the efficacy of current cancer therapeutics, including overcoming the resistance of cancer cells to chemo-radio therapy. However, several clinical and practical challenges need to be addressed before curcumin can be incorporated into regular clinical practice. The purity of the curcumin compound significantly affects its activity, and is of primary importance when used in studies or trials[139]. In addition, body tissue distribution and uptake of curcumin, which account for its biological activity, need better understanding[31]. Clinical trials with curcumin have faced various challenges, such as high metabolic instability, poor aqueous solubility, inadequate focalization, complex pharmacokinetic profile, and poor patient adherence[140].

Nanotechnology-based formulations and analogues have shown potential in overcoming the poor bioavailability issue of curcumin by improving its stability, increasing its cellular uptake, and offering controlled release. However, these formulations often lack tissue specificity. Although the various novel nano-formulations of curcumin show remarkable anti-neoplastic, theranostic, and chemo-adjuvant properties, there are technical challenges in drug development, particularly the need to regulate the size of curcumin nanoparticles for drug delivery applications. In addition, these processes are expensive and have yet to be commercialized. The effects of newer delivery systems, such as polymer nanoparticles and liposomes, on the therapeutic efficacy of curcumin need to be further investigated; while these have been shown to enhance curcumin bioavailability, the possibility of off-target toxicity has not been thoroughly studied[141]. Curcumin has shown cytotoxic and cytoprotective effects at different doses and concentrations in various cancer studies[112,135,142,143]. These findings need consideration in preclinical and clinical trials investigating newer curcumin formulations. Furthermore, the wide range of research variability in human cancer studies using these novel curcumin formulations, such as differences in study design, drug design, sample size, and route of administration, also make it difficult to conclude which formulation has the best overall pharmacokinetic properties[140].

CONCLUSION

In conclusion, encouraging findings from various studies using novel curcumin formulations indicate the need for extensive preclinical and clinical research to shed light on their pharmacokinetics, biocom-

patibility, toxicity, and dose regimens in normal and disease conditions in order to incorporate these agents in cancer treatment strategies. Systematic efforts must focus on identifying a potential curcumin formulation suitable for use in clinical trials. Collaboration between clinicians, translational scientists, medicinal chemists, and pharmacologists is necessary to advance these agents toward clinical use as oral cancer therapeutics.

FOOTNOTES

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ORCID number: Diptasree Mukherjee 0000-0002-8962-2759; Arunkumar Krishnan 0000-0002-9452-7377.

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REVIEW

Evolving utility of exosomes in pancreatic cancer management

Thattungal Manoharan Anoop, Palash Kumar Basu, K Chandramohan, Ajai Thomas, S Manoj

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Thattungal Manoharan Anoop, Ajai Thomas, S Manoj, Department of Medical Oncology, Regional Cancer Center, Medical College Campus, Thiruvananthapuram 695011, Kerala, India

Palash Kumar Basu, Department of Avionics, Indian Institute of Space Science & Technology (IIST), Thiruvananthapuram 695547, Kerala, India

K Chandramohan, Surgical Oncology, Regional Cancer Center, Thiruvananthapuram 695011, Kerala, India

Corresponding author: Thattungal Manoharan Anoop, MBBS, MD, DM, DNB, FRCP Edin, Associate Professor, Department of Medical Oncology, Regional Cancer Center, Medical College Campus, Kumarapuram Road, Thiruvananthapuram 695011, Kerala, India.
dranooptm@yahoo.co.in

Abstract

Despite the development of newer oncological treatment, the survival of patients with pancreatic cancer (PC) remains poor. Recent studies have identified exosomes as essential mediators of intercellular communications and play a vital role in tumor initiation, metastasis and chemoresistance. Thus, the utility of liquid biopsies using exosomes in PC management can be used for early detection, diagnosis, monitoring as well as drug delivery vehicles for cancer therapy. This review summarizes the function, and clinical applications of exosomes in cancers as minimally invasive liquid biomarker in diagnostic, prognostic and therapeutic roles.

Key Words: Pancreatic cancer; Exosomes; Biomarker; Liquid biopsy; Clinical applications; Circulating biomarkers

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Core Tip: The determination and identification of biomarkers using liquid biopsy can enable the early detection, monitoring, therapeutic interventions, risk of relapse, therapeutic targets and identification of resistance mechanisms in pancreatic cancer (PC). There has been a recent interest in use of exosomes as biomarker in PC management. Exosomes loaded with multiple diagnostic molecules can be isolated from different body fluids and can be used for making the exosome markers-based liquid biopsy more attractive for initial tumor detection, monitoring, and prognostic assessment of PC.

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INTRODUCTION

Pancreatic cancer (PC) is associated with poor survival outcome with a 5-year survival of 5%-10%, and a short median survival of 6-8 months after cancer diagnosis[1]. Most individuals diagnosed with advanced disease are symptomatic whereas early stages of the cancer are generally asymptomatic and often undiagnosed. Hence diagnosis is often made after dissemination. Surgery is the only curative treatment[2]. Regrettably, a large number of patients present with unresectable or metastatic disease at the time of diagnosis. Early detection of PC is essential for treatment with curative intent, typically by surgical resection in combination with neoadjuvant or adjuvant chemotherapy and chemo radiation. The majority of patients can have local recurrence or systematic metastasis even after resection. Screening methods for PC often relies on carbohydrate antigen 19-9 (CA 19-9). CA 19-9 demonstrates relatively low sensitivity and specificity in diagnosing PC[3]. Hence a diagnostic test with high sensitivity and specificity and capable of distinguishing PC at early stages from benign diseases is highly recommended. Table 1 shows common circulating biomarkers for PC[4-19]. Comparison of usefulness of various Liquid biopsies used in PC is shown in Table 2[20-30].

BIOGENESIS OF EXOSOMES

There is an emerging role of molecular profiling of liquid biopsies for cancer diagnosis and prognostication. Extracellular vesicles (EVs) derived from various body fluids and serum. There are four subclasses of EV based on different sizes like Exosomes (30-150 nm), Oncosomes (100-1000 nm), Ectoderms (100-1000 nm) and Apoptotic bodies (200-2000 nm). Exosome or Exosomes derived proteins, *etc.* are believed to serve as reliable molecular biomarkers. The circulating vesicles in the blood that originate from tumor cells contains immense proteomic and genetic information to monitor cancer progression, metastasis, and drug efficacy[31-33]. Exosomes were originally introduced during the culture of sheep reticulocytes *in vitro* by Johnstone *et al*[34]. Exosomes are EVs that are endosomal in origin with a diameter of 40-160 nm (average, 100 nm). The formation of cancer cell derived exosomes is depicted in Figure 1. Initially, exosomes are formed by inward invagination of plasma membrane to form an early endosome. These endosomes form nano-sized vesicles resulting in formation of multi vesicular body (MVB) that contain intraluminal vesicles which contain cytoplasmic components including various nucleic acids and soluble proteins[35]. These intra luminal vesicles are released to the extracellular environment by fusing the MVBs with the plasma membrane. Then with the help of exocytosis, exosomes are released in to circulation.

Exosomes contains many molecules like heat shock proteins, RNAs, DNAs, GTPase, CD63, CD81, CD9, CD82, cholesterol, sphingomyelin, and ceramides. Exosomes facilitate both the transport of essential substances like nucleic acids and proteins into various recipient cells and the communications between cells. The main sources of exosomes are plasma, serum, urine, bile, saliva and breast milk. The secreted exosomes have various cellular functions in cell-to-cell interactions and might be pivotal in the occurrence and development of tumour progression and metastasis[36]. Exosomes have definite role in inflammation, coagulation, and embryo implantation in pregnancy. However, cancer cells are capable of secreting 10 times than normal cells. Hence tumour derived exosomes can provide vast information on cancer. Furthermore, exosomes are potential surrogates of the original cells, hence they are useful for understanding cell biology.

Oncosomes are tumor derived cells and they contain different oncogenic molecules that can modify the cells to encourage cancer growth. Tumor cell-secreted exosomes are responsible for paracrine signalling during tumor progression, tumor-stromal interactions, proliferative pathway activation, and immunosuppression[37]. Tumor derived exosomes enters the cells by a variety of methods, depending on the cells that secrete them and the target cells. Metastatic breast cancer derived exosomes use transcytosis to cross the brain endothelial cells, while the "CDC42-dependent endocytic pathway" was utilized to enter astrocytes during brain metastasis[38].

EXOSOMES IN INITIATION OF PC AND METASTASIS

There are increasing evidence that exosomes are involved in the pathogenesis of development of pancreatic inflammation as well as related cancer initiation. Repeated episodes of pancreatitis are a

Table 1 Circulating biomarkers in pancreatic cancer

Biomarker	Type	Role in pancreatic cancer
CA19-9	Protein	Widely used biomarkers to aid in the diagnosis[4]
		Poor screening tool in asymptomatic patients
		Elevated in many benign gastrointestinal conditions as well as other malignancies, including pancreatitis, cirrhosis, cholangitis, and colorectal cancer[5]
		5%-10% of the caucasian population possesses a Lewis a-/b- genotype and thus does not express CA19-9
CEA	Glycoprotein	Elevated across several cancers[6]
		Non specific
		Inferior sensitivity of CEA compared to ca19-9[7]
CA125	Glycoprotein	Associated with ovarian cancer, CRC and cholangiocarcinoma[8]
		Superiority to CA19-9 in predicting resectability of PC, along with correlating with metastasis-associated disease burden
Anti-MUC1 antibody	Antibody	Anti-MUC1 antibody assays showed a sensitivity and specificity of 77% and 95%, respectively, in discriminating pancreatic cancer from pancreatitis[9]
CTCs	Tumour cells	CTCs had moderate diagnostic value in PC[10]
		Several studies have demonstrated isolation of CTCs regardless of stage among localized, locally advanced, or metastatic patients
		Conflicting evidence on CTC positivity is correlated with survivability
		In ombination with CA19-9, it was reported to have a superior sensitivity and specificity of 97.8% and 83.3% respectively, compared to when used in isolation[11]
		The presence of CTCs in 54/72 patients with confirmed PDAC (sensitivity = 75.0%, specificity = 96.4%, AUROC = 0.867, 95%CI: 0.798-0.935, and $P < 0.001$)[12]
		A cut-off of ≥ 3 CTCs in 4 mL blood could differentiate between local/regional and metastatic disease (AUROC: 0.885; 95%CI: 0.800-0.969; and $P < 0.001$)
cfDNA	DNA	Plasma ctDNA quantification of hot-spot mutations in KRAS and GNAS are useful in predicting tumor burden in patients diagnosed with PC[13]
		Digital PCR provided accurate tumor-derived mutant KRAS detection in plasma in resectable PC and improved post-resection recurrence prediction compared to CA19-9[14]
		Detection of plasma cfDNA mutations and copy number alterations may be helpful in pancreatic cancer prognosis and diagnosis
		Its sensitivity and specificity in identification of clinically relevant KRAS mutations was 87% and 99% respectively[15]
Cell-free RNA	RNA	Higher expression of lncRNA MALAT1 has been shown to correlate with poorer PDAC survival[16]
		Several microRNAs have also been associated with PDAC (*i.e.*, miR-21 and miR-155), and correlate with tumor stage or prognosis[17]
EVs	Exosomes	KRAS G12D mutations were identified in 7.4% of control patients, 67% of localized PDAC, 80% of locally advanced PDAC, and 85% of metastatic PDAC patients[18]
		GPC1 EVs could be detected in both pancreatic precursor lesions and pancreatic cancer, and could distinguish between any evidence of malignancy and healthy patients with an AUC of 1 (100% sensitivity, 100% specificity)[19]
		miRNA isolated from EVs revealed a cocktail of miRNAs (miR-1246, 4644, 3976, 4306) upregulated in 83% of pancreatic cancer derived EV
		Glypican-1 exosomes are a potential biomarker for PC

CA: Carbohydrate antigen; PDAC: Pancreatic ductal adenocarcinoma; PC: Pancreatic cancer; CEA: Carcinoembryonic antigen; CTCs: Circulating tumour cells; cfDNA: Cell-free DNA; EVs: Extracellular vesicles; AUROC: Area under the curve.

strong risk factor which can eventually increase the risk of PC. The pathogenesis and evolution of many pancreases precancerous conditions, including diabetes mellitus and pancreatitis, have been linked to crucial involvement of exosomes[39]. Exosomes can participate in promoting the transformation of various precancerous like intraductal papillary malignant neoplasm to PDAC. Exosomes are a key factor in initiating angiogenesis, cell migration, and epithelial-mesenchymal transition[40]. Cancer-associated fibroblasts, tumor-associated macrophages and pancreatic stellate cells can promote exosomes, that could promote growth, proliferation, drug resistance, epithelial mesenchymal transition, migration, invasion and metastasis of PC[41]. Interestingly, exosomes initiated from PC cells contains

Table 2 Comparison of usefulness of various liquid biopsies in pancreatic cancer

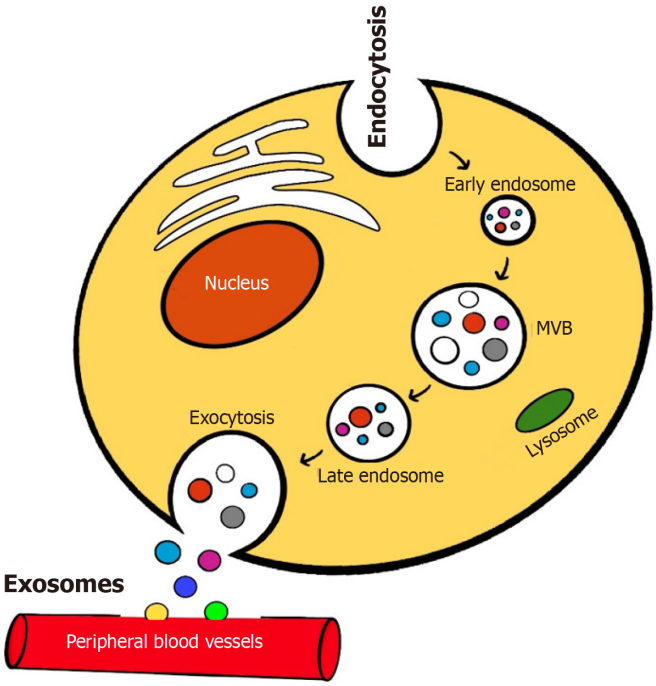
Item	CTC[20-22]	Ct DNA[21,23,24]	Exosomes[20,25-28]	CA 19-9[20,21,28-30]
Origin	Viable tumor cells	cfDNA, viable tumor cells, CTCs	DNA, proteins, lipids, RNAs metabolites, and tumor cells	Ductal cells in the pancreas, biliary system, and epithelial cells in the stomach, colon, uterus, and salivary glands
Samples used	Plasma	Frozen plasma, urine and other biofluids	Frozen plasma, urine and other biofluids	Plasma
Methods	CellSearch, MACS, Dynabeads, microfluidic, SE-iFISH, CD45/CEP8/DAPI staining-FISH, anti-EpCAM Portal-vein blood	Real-time quantitative PCR, digital PCR, droplet digital PCR, next-generation sequencing; commercial liquid biopsy platforms: GuardantTM (breast, colon, and lung cancers and multi-cancer detection) FoundationOne® (multi-cancer detection); signateraTM (colorectal cancer), Galleri (multi-cancer detection), CancerSEEK (multi-cancer detection), TempusTM (multi-cancer detection), Caris (bioinformatics testing of both circulating DNA and RNA)	Ultracentrifugation, ExoChip, precipitation, size-based isolation immunoaffinity-based isolation microfluidics-based isolation	Radio immuno assay
Mutation analysis	Yes	Yes	Yes	No
Drug delivery vehicle	No	No	Yes	No
Sensitivity	76.0%	65.0%	50.0%-85.0%	78.2%
Specificity	68.0%	75.0%	90.0%	82.8%
Usage in clinics	Diagnosis of PDAC, prognosis/prediction of PDAC	Diagnosis of PDAC; monitoring treatment efficacy; monitoring of disease progression	Diagnosis and prognosis of PDAC; prognosis/prediction of PDAC	Combining ct DNA with CA 19-9 levels could improve diagnostic sensitivity to 98%, and specificity to 97%; monitoring treatment efficacy; monitoring of disease progression

PDAC: Pancreatic ductal adenocarcinoma; CTC: Circulating tumour cells; CA: Carbohydrate antigen; ctDNA: Circulating tumor DNA.

tumor suppressor components which can inhibit the cancer cell proliferation and this could open the pandora box of potential therapeutic value of exosomes[42]. Exosomes promote cancer cell proliferation and initiate metastasis by delivering carcinogenic proteins, cytokines, adhesion molecules and miRNA. Thus, initiate proliferation of tumour by activation of different pathways like phosphoinositide 3-kinase/AKT serine/threonine kinase 1 (Akt) and mitogen-activated protein kinase pathways[43]. The features like weight loss and new-onset diabetes are characteristics of the paraneoplastic effect of PC which mostly precede the diagnosis of the PC. The biological reason of PC-associated diabetes is due to exosomal adrenomedullin, endoplasmic reticulum stress which may result in β-cell dysfunction and diabetes[44]. There is emerging evidence that suggest role of exosomemediated immunosuppression in PC. The exosomes have clear role in communications between tumor and immune cells and supposed to have a dynamic role in tumor immunity regulation. Gemcitabine chemotherapy is considered a standard treatment for PC either in combination or monotherapy, based on evidence from many studies which shown a better survival rate and more clinical benefits with median overall survival (OS) of 5 mo to 7 mo[45]. Most of patients with PC ultimately present with rapid disease progression even following chemotherapy with gemcitabine. Tumor derived exosomes can induce the progression of chemotherapy resistance in cancer cells. Chemotherapy agents could also be secreted from the extracellular matrix by exosomes is another reason for chemotherapy resistance. When exposed to gemcitabine, exosomal CAFs which are inherently insensitive to gemcitabine may also leads to chemotherapy resistance. CAF exosome secretion inhibition could decrease proliferation and drug resistance[46].

ISOLATION OF EXOSOMES

There are various methods to isolate and characterize exosomes based on their physical and chemical properties. Most popular methods are ultracentrifugation, size exclusion chromatography, magnetic activated cell sorting, membrane filtration and various commercial kits[47]. Western blotting and flow cytometry can be used to analyze and detect exosome markers. Transmission electron microscopy and



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Figure 1 Diagrammatic representation of formation of cancer cell derived exosomes. MVB: Multi vesicular body.

nanoparticle tracking analysis are other methods to detect exosome.

Liquid biopsy to analyze exosome biomarkers could guide the diagnosis and prognosis of PC. Therefore, the identification of reliable predictive biomarkers for diagnosis and prognosis is an unmet need in PC management. The most common methods for isolation for exosome are summarized in Table 3[48-56] and quantifying methods for exosome are presented in Table 4[48-64]. Methods like Western blotting and enzyme linked immunosorbent assays needs large amounts of sample and extensive technical steps for detection. The comparison of various isolation methods used for exosomes are given in Table 5[65-76].

EXOSOMES AS DIAGNOSTIC BIOMARKER IN PC

At present scenario, early diagnosis of PC is very difficult and most are diagnosed at late stage. Mostly CT imaging are used for diagnosis and treatment. CA19-9 which is used in clinical practice has a low specificity and poor ability to distinguish benign pancreatic diseases from PC[77]. Thus, the search for novel early diagnostic markers is a concern for PC diagnosis and treatment. Exosomes are excessively produced in excess by malignant tumours. They also carry information about the tumour genetics and microenvironment, which determines its behaviour and its prognosis[78]. Circulating biomarkers are non-invasive and inexpensive for monitoring disease[79]. The circulating molecular tumor markers are circulating tumor cells, cell-free DNA, cell-free RNA, circulating tumor proteins, and exosomes. When compared to circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA), exosomes are considered as the best diagnostic biomarkers in PC with a sensitivity of 93% and a specificity of 92% [80]. The ctDNA group had similar specificity 0.92 (95%CI 0.88-0.95) but lowest sensitivity. Thus, ctDNA was useful for the diagnosis of PC rather than screening. Whereas CTCs exhibits medium sensitivity and lowest specificity compared to others. The sensitivity, specificity and AUC of ctDNA were 0.6400, 0.9200, and 0.9478 respectively. Glypican 1 (GPC1) is expressed in the serum of patients with PC but not in benign pancreatic disease. Melo *et al*[19] described that Glypican-1 identifies cancer exosomes and could detects early PC. It was described that GPC1+ circulating exosomes could be used as prognostic biomarker in pre- and post-surgical patients. The GPC1+ circulating exosomes could distinguish PC precursor lesions from healthy individuals and benign pancreatic disease. The circulating GPC1+ exosomes levels were higher in PC precursor lesions than the levels in the healthy donor group and benign pancreatic diseases. There is supporting evidence that there is a potential use in early detection of pancreas cancer. Melo *et al*[19] study shows that circulating GPC1+ exosomes exhibit a sensitivity of 100% and specificity of 100%; with a positive predictive value of 100% and a negative predictive value of 100% compared to CA 19-9 which was inferior in distinguishing between pancreas cancer and healthy controls.

Table 3 Different isolation methods for exosome

Method	Sample volume	Time	Ref.
Ultracentrifugation	Low	Approximately 5 h	[48,49]
Density-gradient	Low	Approximately 5 h	[50]
Nanopillar	30 μL	Approximately 10 min	[51]
Acoustic-based	0.4-0.7 μL/min	< 30 min	[52]
Inertial lift force-based	70 μL/min	> 4 h	[53]
Surface-modified	4-16 μL/min	< 1 h	[53-55]
Nanoshearing	Not mentioned	< 3 h	[56]

Table 4 Different quantifying methods for exosome

Method	Size range	Specificity	Time	Ref.
Nanoparticle tracking analysis	10 nm-2 μm	Immunoaffinity	< 1 h	[48]
Dynamic light scattering	10 nm-8 μm	Size	< 1 h	[57]
Electron microscopy	10 nm	Size	< 1 h	[58,59]
Nanopore	> 10 nm	Size	< 1 h	[60,61]
Magnetic resonance	Wide range	Immunoaffinity	< 10 min	[62]
Electrochemical and plasmonic	Depends on binding	Immunoaffinity	< 10 min	[63,64]

The functional role of MicroRNAs has a greater opportunity in developing both prognostic and diagnostic markers. MiRNA -based biomarkers can help in the early diagnosis of disease. A recent study evaluated the expression patterns of miR-21, miR-155, miR-17-5p, and miR-196a in circulating exosomes as biomarkers for PC. The expression profile of miR-17-5p and miR-21 were increased in PC patients, The increased expression of miR-17-5p was seen in unresectable pancreatic patients[81]. miR-155 and miR-21 are over-expressed in PDAC, and can distinguish PC from benign lesions[82]. Upregulated miR-221-3p and miR-212 is associated in PDAC and they are responsible for cancer proliferation in PDAC cells. miR-128 expression is decreased in PC while non-cancerous tissue has a normal level of miR-128. Gemcitabine resistance is associated with downregulation of miRNA200b, miRNA-200c, let-7b, let-7c, let-7d, and let-7e. miR-155 and miR-1246 also have been related to gemcitabine resistance. There are miRNA that function as tumor suppressors in pancreatic ductal cancer like miR-99b, miR-100, miR-99a, miR-34a, miR-148a, miR-200a, miR-200b, and miR-200c. MicroRNAs expression profiles showed that miR-143, miR-29c, miR-148b, miR-150, and miR-96, were present in PDAC and chronic pancreatitis whereas miR-196b, miR-203, miR-196a, miR-210, miR-222, miR-210, miR216, miR-375, and miR-217, were expressed only in pancreatic carcinoma[83]. miR-190, miR-196a, miR-222, miR-15b, miR200b, miR-95, and miR-221 are elevated in pancreatic adenocarcinoma[84]. Nakamura *et al*[85], developed an exosome-based signature for non-invasive and early detection of PDAC. Previous research studies showed that serum Ephrin type-A receptor 2 in exosomes (Exo-EphA2) was expressed highly in PC cells. A study by Wei *et al*[86] the evaluated role of serum Exo-EphA2 as a potential diagnostic biomarker in PC. Serum Exo-EphA2 were higher in PC than in non-cancer pancreatic disease. Exo-EphA2 in combination with CA 19-9 was more useful to discriminate early stage of PC from non-cancer pancreatic disease. Alkaline phosphatase placental-like 2 presents in PC EVs has a potential application in liquid biopsy-based diagnostic tests. Shin *et al*[87] developed ALPPL2 direct and sandwich aptamer-linked immobilized sorbent assay for EVs, which could sensitively and specifically detect membrane protein,17 could be a potential biomarker for early diagnosis of PDAC. Recently, there are reports of exosomal migration inhibitory factor (MIF) may be an attractive sensitive biomarker for PC. MIF is an immunostimulatory cytokine associated with tumorigenesis. Costa-Silva *et al*[88] reported that the pancreatic exosomes are capable of inducing premetastatic niche formation in liver. They demonstrated that the exosome education-induced liver metastasis was abolished by silencing of exosomal MIF. The combined use of exosomal glypican-1 and MIF is a promising tool to identify very early stages of PC. The potential of MIF as a target for the treatment of PDAC should be explored in future.

Table 5 Comparison of various isolation methods for exosomes

Conventional isolation of exosomes				
Methods	**Advantages**	**Disadvantages**	**Clinical use**	**Ref.**
Ultracentrifugation	Widely used; high purity; protein and RNA components are not affected	Highly labour intensive; time-consuming; yields are typically low extensive training of personnel needed; expensive; inappropriate for the extraction of exosomes from a small amount of serum samples	Functional study of exosomes	[65, 66]
Ultrafiltration	High yield; simple; less time-consuming; do not require the use of special equipment	Low purity, clogging of pores	Study of sample concentration; used in combination with other methods	[67]
Precipitation	Widely used; economical	Co-isolation of non-EV particles	For studies with very low purity requirements that do not require omics studies	[68]
Size exclusion chromatography, OR, and gel filtration	Fast, reliable, and inexpensive; maintain the biological activity and integrity of exosomes; high purity	Nanoscale contaminants like lipoproteins; extensive laboratory equipment requirements	Suitable for exosome research in those requiring high purity, omics, and large volume samples	[69]
Immunoaffinity capture	Convenient; not affected by exosome size; no need for expensive instruments	Expensive; low capacity; low yields	Suitable for the Separation of specific exosome subgroups	[70]
Emerging isolation methods				
Stirred ultrafltration	Do not rely on equipment; less time consuming; reduces the destruction of exosomes during the process	Moderate purity of isolated exosomes; loss of exosomes during the process	Isolating exosomes from culture supernatant of bone marrow mesenchymal stem cells	[71]
ExoTIC (exosome total isolation chip)	Simple, easy-to-use, modular, and facilitates high-yield and high-purity EV isolation from biofluids	Special equipment requirements; lack of tests on clinical samples	Efficiently isolate EVs from small sample volumes; EV-based clinical testing from fingerprick quantities (10-100 μL) of blood	[72, 73]
3D ZnO Nanoarrays	Multifunction; high sensitivity; downstream analysis is possible; enhance the capture of exosomes at a high flow rate	Relatively expensive	Widely used in biosensing and analysis aspects, powerful tools for effective purification and molecular analysis of exosome	[74, 75]
Nano plasmon-enhanced scattering	Rapid, high-throughput, sensitive, and specifc method for the detection of exosomes from trace samples depending on the amount of scatter area, based on calculation of the proportion of the area that contains scattered light	High reagent cost; complex statistical tools; low capacity	Uses antibodies against the cellular markers CD81, CD63, and CD9, which are enriched on most exosome membranes	[76]

SEC: Size exclusion chromatography; EV: Extracellular vesicle.

EXOSOMES AS PROGNOSTIC BIOMARKER IN PC

The level of circulating Exo-EphA2 was higher in PC patients when compared to that of healthy controls, suggesting it could be a diagnostic and prognostic marker for PC. In a study by Wei *et al*[86] found that high expression of Exo-EphA2 in PC was associated with shorter OS. Exosomal KRAS mutations seems better than CA 19-9 Levels for the prognostic surveillance in PDAC[17]. A study by Tsuchida *et al*[89] revealed that KRAS mutation detected at baseline with a mutation frequency above 5% indicated poor clinical outcome following monitoring in the treatment course of patients with metastatic PDAC. Costa-Silva *et al*[88] found that MIF was markedly higher in exosomes from stage I PDAC patients who later developed liver metastasis. Thus, it is suggested that higher exosomal MIF may be a prognostic marker for the development of PDAC liver metastasis. Potential role of exosomal biomarkers for prognosis evaluation in PDAC was evaluated in a systematic review and meta-analysis, involving eleven studies comprising 634 patients and seen that detection of positive exosomal biomarkers increased risk of mortality and progression across disease stages. Positive exosomal biomarkers preoperatively had higher risk of mortality in resectable stages than positive exosomal biomarkers in unresectable stages[90].

The better understanding of the prognostic role of miRNAs in PC can be done by profiling miRNAs at different stages of cancer. In a study by Takahasi *et al*[91], authors suggest that plasma exosomal miR-451a may be useful to predict recurrence and prognosis in PDAC patients. The miR-451a had a significant association with tumor, stage and showed the highest upregulation in the stage II patients

who showed recurrence after surgery. In a retrospective clinical study by Namkung *et al*[92] comprising 200 pancreatic ductal adenocarcinoma tissue samples, miRNA-574-5p, miRNA-1244, miRNA-145, miRNA-328, miRNA-26b, and miRNA4321 showed association with OS and disease-free survival. Poor survival outcomes have been seen in PDAC with lower expression of *miR-183* and *miR-34a* as well as high expression of *miR-1290*, *miR-155*, *miR-203*, *miR-222*, and *miR-10b*[93]. Similarly, Microarray-based expression profiling of miRNAs derived from exosomes study revealed that miR-451a was the highest upregulated miRNA in stage II patients who developed recurrence after surgery. It was seen that survival rates of the high exosomal miR-451a patients were significantly worse than those of the low miR-451a patients[94].

TREATMENT MONITORING IN PC

Currently, one of the most common biomarkers used for so long to monitor the therapeutic responses in PC is CA 19-9. Exosomes have a significant role in monitoring response to therapy and disease progression. Melo *et al*[19] clearly demonstrated that all 190 patients with PDAC serum had higher GPC1+ exosomes than healthy individuals and was an independent prognostic marker for disease specific survival. In view of the fact that CA19-9 is not a reliable marker that correlates with clinical evolution of PC, a combination of CA19-9 together with exosome derived GPC1 could be explored for treatment monitoring and disease progression. Besides early diagnosis and prognosis, clinical utility of exosome proteins is evolving for personalized and posttreatment disease monitoring[95]. Circulating exosomal PD-L1 is an attractive option in disease monitoring. Recently, Chen *et al*[96] study explains the rationale for the application of exosome PD-L1 as a predictor for anti-PD-1 therapy.

EXOSOMES AS DRUG CARRIERS, THERAPEUTIC TARGETS AND TREATMENT

Currently, innovators are exploring the utility of exosomes for biomedical applications. Many advanced drug delivery systems that used to deliver various anticancer and antiviral agents explore the use of polymeric nanoparticles and liposomes to encapsulate drug and thus utilize for drug delivery.

Exosomes can be used as therapeutic drugs carriers because of favourable bioavailability, biocompatibility, ability to penetrate biological membranes and immunogenicity[97]. Exosomes can be used as transporters, therapeutic targets and therapeutic drugs.

Due to the favorable bioavailability and biocompatibility with the characteristics of exosomes, there appears a greater future of exosomes used either as parental exosomes or artificially modified exosomes for drug delivery vehicle. To avoid systemic toxicity, drugs can be encapsulated in exosomes and transferred to target cells[98]. Exosomes possess better biocompatibility as drug carriers. It is generally considered that injected exosomes shed from endogenous cells are tolerated with minimal immune reaction. The cargos can be delivered into the tumor microenvironment with the utility of exosomes [99]. Kamerkar *et al*[100] studied modified exosomes for cancer prevention and treatment and revealed that exosomes had a longer retention time in the circulation. Engineered exosomes specialized for malignant KRAS G12D were more successful in targeting oncogenic KRAS. Recent evidence suggests that safety and efficacy of exosomes in treating PC is not far. Exosome-based therapies for cancers have been developed due to the easy permeability of the exosome membrane, low toxic side effects and low immunogenicity. Paclitaxel -loaded exosomes have shown a great potential for delivery of chemotherapy and treatment of drug-resistant cancers[101]. Because of their rapid clearance from blood circulation after systemic administration, targeted delivery of exosomes is highly restricted. The rapid clearance after injection limits their applications for effective and durable therapeutic action. However, recent studies on modification of exosomes for targeted delivery *via* direct modification and genetic engineering to circumvent this limitation is promising. The use of MSCs-derived exosomes loaded with KRAS G12D siRNA to treat metastatic pancreas cancer (NCT03608631) is promising[102]. Mittal *et al* [103] also showed the efficacy of administration of micelles of gemcitabine and the tumor suppressor *miRNA-205* for the treatment of pancreas. Masamune *et al*[104] found that hypoxic environment in PC can release several angiogenic factors that may induce proliferation and angiogenesis. Understanding of these interactions under hypoxia is critical for angiogenic regulation in PDAC, which will also help to develop new anti-angiogenesis therapeutic strategies[105].

CONCLUSION

Even though there are several limitations in implementing exosome analysis clinically, it is a promising diagnostic and therapeutic tool for PC. The role of exosomes in cancer treatment continues to evolve.

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FOOTNOTES

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Country/Territory of origin: India

ORCID number: Thattungal Manoharan Anoop 0000-0003-0567-5253.

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MINIREVIEWS

Adult eosinophilic esophagitis and advances in its treatment

Martina Grando, Silvia De Pauli, Giovanni Miotti, Massimiliano Balbi, Marco Zeppieri

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Martina Grando, Massimiliano Balbi, Department of Internal Medicine, Azienda Sanitaria Friuli Occidentale, San Vito al Tagliamento 33078, Italy

Silvia De Pauli, Department of Internal Medicine, Azienda Sanitaria Friuli Occidentale, Pordenone 33170, Italy

Giovanni Miotti, Department of Plastic Surgery, University Hospital of Udine, Udine 33100, Italy

Marco Zeppieri, Department of Ophthalmology, University Hospital of Udine, Udine 33100, Italy

Corresponding author: Marco Zeppieri, BSc, MD, PhD, Doctor, Department of Ophthalmology, University Hospital of Udine, p.le S. Maria della Misericordia 15, Udine 33100, Italy. markzeppieri@hotmail.com

Abstract

Eosinophilic esophagitis (EoE) is a chronic eosinophil inflammation that seems to be T helper type 2 antigen-driven. The disease is one of several eosinophilic gastrointestinal disorders in which there appears to be inflammation of the gastrointestinal tract without any apparent underlying causes. Differential diagnosis needs to be made with gastroesophageal reflux, which is characterized by chronic inflammation due to gastric refluxate from disorders related to motility. EoE, however, is considered a chronic allergic inflammatory disorder related to destructive tissue remodeling. There seems to be a higher prevalence of EoE in Western countries. It is typically found in atopic male individuals. Physiopathological risk factors include atopy, environmental factors, esophageal epithelial barrier dysfunctions, *etc.* EoE can cause several symptoms that include retrosternal burning sensation, dysphagia, food impaction, chronic reflux symptoms, nausea, and vomiting. Early diagnosis, which requires a biopsy to assess for esophageal inflammation, is essential for proper treatment. The aim of our brief overview is to summarize the current literature regarding the characteristics, diagnosis, complications, mechanisms of pathology, clinical features, influence of comorbidities, and treatment in patients with EoE.

Key Words: Eosinophilic esophagitis; Gastroesophageal reflux; Chronic allergic inflammatory disorder; Eosinophil inflammation; T helper 2

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Core Tip: Eosinophilic esophagitis (EoE) is a chronic eosinophil inflammation. Differential diagnosis needs to be made with gastroesophageal reflux, which is characterized by chronic inflammation due to gastric refluxate from disorders related to motility. It is of clinical importance to diagnose, manage, and treat individuals with EoE. Patient outcomes, success of therapy, prevention of complications, and management of existing comorbidities depend on proper organ functioning.

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INTRODUCTION

Eosinophilic esophagitis (EoE) can be characterized as a T helper type 2 (Th2) antigen-driven disease that is due to chronic eosinophil inflammation, which causes signs and symptoms of esophageal dysfunction. The disease is part of a spectrum of eosinophilic gastrointestinal disorders that show eosinophilic inflammation of the gastrointestinal tract that can be explained by other causes. The first cases were described in the 1970s. Before 1960, interrogation of the intestinal mucosa was limited to surgical resections or postmortem analyses. An important element in the study of this disease was the advent of luminal fiberoptic endoscopy[1].

Esophageal eosinophilia was believed to be solely a manifestation of reflux esophagitis[2] and EoE was seldom diagnosed. In the mid-1990s, however, several studies described the disease as it is recognized today[3,4]. Since then, the number of publications on EoE has increased dramatically, especially considering that this condition has become more prevalent over the past 20 years as one of the main causes of upper gastrointestinal disorders. With the current diagnostic technology available, it is possible to provide a better diagnosis of gastroesophageal reflux (GERD) and EoE. GERD is characterized by chronic inflammation resulting from exposure to luminal gastric refluxate deriving from a disorder of motility. EoE, however, is defined as a chronic allergic inflammatory pathology characterized by signs, symptoms, and complications which tend to be related to destructive tissue remodeling [1].

The incidence rates of EoE continue to rise[5]. The prevalence of EoE is increasing rapidly in modern times, with rates approaching the prevalence of inflammatory bowel diseases. The prevalence of EoE follows the rising trend of allergic diseases over the last 50 years[6]. The prevalence estimates of EoE vary in different parts of the world. Studies have demonstrated a high prevalence in Western countries and a low prevalence in Eastern countries[5]. EoE occurs both in the pediatric and adult populations and tends to be more common in atopic male patients. Approximately 75% of all EoE patients tend to be male. This increased gender risk of EoE is supported by a genetic variant in the cytokine receptor-like factor 2 gene that encodes for the thymic stromal lymphopoietin receptor[6].

The aim of our minireview is to briefly assess the current literature regarding the characteristics, diagnosis, complications, mechanisms of pathology, clinical features, influence of comorbidities, and treatment in patients with EoE that have been published in the literature and considered in clinical settings since 2000. We conducted a search of the published literature from January 1, 2000 to March 1, 2023, using PubMed (https://pubmed.ncbi.nlm.nih.gov) and *Reference Citation Analysis* (*RCA*) (https://www.referencecitationanalysis.com). The main topics in this minireview can be found in Table 1.

PHYSIOPATHOLOGY

Atopy

EoE is considered an atopic disease, which is associated with food antigen-driven hypersensitivity. The majority of patients with EoE have an atopic phenotype. Individuals with EoE tend to have higher rates of other allergic conditions such as asthma, atopic dermatitis, allergic rhinitis, or increased IgE to aeroallergens and foods[6,7].

Environmental factors

Studies have shown that certain environmental factors can increase the risk of EoE, which include: Dysbiosis and dysregulation of the microbiome; residence in rural and suburban areas; cesarean birth; exposure to antibiotics; lack of breastfeeding, *etc*[8].

Table 1 Main topics of the minireview

Main topic	
Physiopathology	Atopy
	Environmental factors
	Esophageal epithelial barrier
	Th2
	Eosinophils
Clinical presentation	
Diagnosis	
Treatment	Dietary management
	Pharmacologic therapy
	Esophageal dilatation
	Biologic agents
Conclusions	

Th2: T helper type 2.

Esophageal epithelial barrier

The epithelium plays a central role in the development of EoE. In this condition, the epithelium demonstrates characteristic alteration that includes basal cell hyperplasia, dilated intra-cellular spaces, and impaired barrier function. When the epithelium barrier is broken or disrupted, it can lead to a hypersensitivity immune response to foreign antigens[6]. Several EoE patients can have an altered epithelial barrier when there are no signs of inflammation, which can predispose these individuals to allergic sensitization. Studies have reported the presence of transcriptional alterations in individuals with EoE[1]. There seems to be a downregulation of genes including filaggrin and involucrin. Other junctional proteins like claudin-1 and E-cadherin can also be decreased in EoE. Deoxyspergualin (DSG) (a transmembrane desmosomal cadherin) shows decreased activity in individuals with EoE[6]. Calpain 14 (CAPN14) has been found to be the most highly associated gene with EoE[9,10]. It is overexpressed in the esophageal epithelium in patients with EoE. Induced interleukin (IL)-13 stimulation in the esophageal epithelium can cause overexpression of CAPN14 and impaired barrier functioning. Altered expression of these genes and/or activation by type 2 cytokines such as IL-13 may predispose to barrier dysfunction.

The alteration of the barrier can also be due to peptic or other injuries. This hypothesis is derived from the clinical signs of EoE that can form after epithelial damage from trauma, acid, or infection. In these circumstances, food and other substances in direct contact with the damaged epithelium can sensitize the microenvironment of the esophageal mucosa and lead to activation of the Th2 inflammatory response. Barrier dysfunction can also occur as a self-perpetuating product of inflammation. Once the inflammation starts, the epithelium can become increasingly permissive and allow more allergenic stimulation to penetrate and develop an ongoing allergic cycle[1].

Th2

EoE is also Th2-mediated. The cascade of inflammatory response is similar to that of chronic allergic disease with an aberrant Th2 response. The lymphocytes are cells typically present in the inflammatory infiltrates found in EoE[11]. This immune response is mediated by ILs secreted by Th2, like IL-4, IL-5, and IL-13.

IL-5 is secreted by mast cells, Th2 cells, and eosinophils. This substance influences eosinophil survival and expansion and primes them to respond to activating signals in chronic allergic reactions[6]. IL-13 is one of the most important cytokines involved in EoE pathogenesis. Studies have demonstrated that IL-13 is upregulated in the esophagus of patients with EoE[12]. It can induce remodeling in the esophageal epithelial barrier; it has been shown to downregulate DSG-1, filaggrin, and involucrin[13]. IL-13 also upregulates eosinophil chemotaxis inducing the expression of chemokine ligand 26. Furthermore, it is responsible for epigenetic modification in the expression of CAPN14, an esophageal-specific protease [14]. CAPN14's substrates include signaling molecules, structural proteins, cell adhesion molecules, transcription factors, and inflammatory mediators of allergic responses, like IL-33 and STAT6[12], both of which tend to be pivotal in the development of EoE.

Eosinophils

Under normal conditions, the human esophageal epithelium has little or no eosinophilic leucocytes. The presence of intraepithelial eosinophils in the esophageal lamina propria and submucosa defines EoE [15]. Eosinophils are recruited by local chemotaxis. They have been shown to have effects on tissue damage[16]. Eosinophil also acts as antigen-presenting cells, recruiting T cells, mast cells, and basophils.

CLINICAL PRESENTATION

EoE may present with a wide variety of symptoms, which ranges from retrosternal burning sensation to dysphagia or episodes of food impaction. Some patients complain of chronic reflux symptoms, nausea, and vomiting. The clinical presentation tends to be different between adults and children[17,18]. The most typical signs and symptoms in adults include heartburn, dysphagia, chest pain, and food impaction. About 50% of adult patients initially presenting with food impaction have been shown to have a diagnosis of EoE[19]. In contrast, children present more commonly with vomiting, regurgitation, abdominal pain, and failure to thrive. Numerous patients begin to implement compensatory behaviors, such as eating food cut into small pieces, eating slowly, lubricating items with sauces, chewing carefully, diluting foods with drinking liquids, and avoiding medications and substances that induce dysphagia[8]. Several validated tools are now available to evaluate symptoms. In adults, the Eosinophilic Esophagitis Activity Index has been shown to offer good correlations between symptoms, histology, and patient-reported outcomes[20]. The most serious complication of EoE is the spontaneous rupture of the esophagus after a food impaction or episodes of vomiting (Boerhaave's syndrome). Fortunately, this complication is rare. Other associated complications that can happen in association with EoE include esophageal stricture, perforation, food impaction, and malnutrition[8].

DIAGNOSIS

There is no specific or diagnostic symptom of EoE. Diagnosis normally requires a biopsy to assess for esophageal inflammation. Endoscopic features can be misleading, thus several esophageal biopsies are needed in all individuals with risk or suspicion of EoE independent of endoscopic clinical appearance [21]. The most typical endoscopic signs in adults with EoE include mucosal rings (64%), linear furrows (80%), white plaques and/or exudates (16%), small caliber esophagus (28%), and strictures (12%)[22]. An endoscopic scoring system has recently been developed and validated to assist in the assessment and standardization of EoE signs based on the presence of rings, edema, exudates, strictures, and furrows[23].

The presence of the following criteria is required for the diagnosis based on the American College of Gastroenterology Clinical guidelines and consensus recommendations, which were reported in a review study of EoE by Gomez Torrijos *et al*[24]: (1) Clinical symptoms of esophageal dysfunction; (2) An increased number of eosinophils in the esophageal epithelium, with ≥ 15 eosinophils per high-power field, and the eosinophilia is limited to the esophagus; and (3) Exclusion of other possible causes of esophageal eosinophilia (including eosinophilic gastroenteritis, infection, hypereosinophilic syndrome, *etc*.). Characteristic histological features, which, however, are not pathognomonic, are also eosinophil aggregates or micro-abscesses and eosinophil layering along the surface of the lumen[8].

TREATMENT

The goals of the therapy of EoE are to induce histological remission, improve clinical symptoms, and prevent disease progression and complications. The management of EoE includes dietary, pharmacologic, and endoscopic interventions[8]. Commonly used treatments include an elimination diet, acid suppression with proton-pump inhibitors (PPIs), topical steroids, and, in the case of strictures, esophageal dilatation. New therapeutic options include monoclonal antibodies that are tailored to stop the underlying inflammatory processes. Increasing evidence suggests that type 2 cytokines may play key roles in EoE[25].

Dietary management

Diet therapy may prove to be very effective and can directly assist in the underlying pathological allergic mechanism. This method can also assist to identify the various food antigens that are responsible for the inflammatory response[8]. In patients with EoE, allergy tests to identify food allergens that can contribute to the pathogenesis of the disease can be useful to initiate a specific elimination diet. The main methods for testing food allergies are the skin prick test (SPT), *in vitro* specific IgE testing, and patch test (used to identify non-delayed allergic reactions). When an allergy test identifies a specific food allergy, the initial treatment is a testing-directed elimination diet. Studies in

adults and children have shown that direct diets based upon *in vitro* specific immunoglobulin E testing and SPT/patch testing can be successful to varying extents, although further studies are needed before routine use of serum-based testing can be recommended[26,27]. The patient should be referred to an allergology specialist to evaluate the prescription of epinephrine for anaphylaxis self-treatment.

Other dietary approaches are the empiric elimination diet which consists in educating the patient to avoid foods that are most likely to be allergenic (*i.e.*, SFED or the six-food elimination diet which is based on eliminating egg, cow milk, wheat, soy, fish/ shellfish, and peanuts/tree nuts). An elemental diet is an amino acid-based formula with the exclusion of all solid foods[28].

All these diets last normally for several weeks (at least eight weeks). The clinician must then check symptoms and confirm histologic improvement with esophageal biopsies. Problem foods are then slowly reintroduced one at a time, with a periodic evaluation to determine if they are tolerated.

Several prospective adult studies based on an elemental diet showed a lower histologic response in about 3 of 4 cases; however, these trials were limited by a short treatment duration of 4 wk and relatively high dropout and nonadherence due to palatability[28]. A meta-analysis, however, reported better clinical outcomes with the elemental diet when compared with specific food elimination diets [29]. Nonadherence to an elemental diet is due to limited meal variety, taste, lack of insurance coverage, and numerous endoscopies needed during food reintroduction to identify specific triggers[30].

Pharmacologic therapy

PPIs: PPIs are the first-line treatment options with dietary modification and topical glucocorticoids. *In vitro*, studies have shown that PPIs decrease cytokine secretion from the esophageal epithelium, which leads to the idea that PPIs can give an anti-inflammatory benefit[31]. The reported response rates to PPI therapy in patients with EoE can vary widely, ranging from 30%-70%[21]. There is no specific element that can predict the patient's response to these drugs. PPIs may benefit EoE patients by reducing acid production in patients with coexistent GERD. Individuals with well-established EoE can also have symptoms of GERD that are responsive to PPI treatment, where GERD can contribute to the development of EoE[8].

Topical glucocorticoids: Current steroid formulations used in the treatment of EoE are designed for airway delivery. There are various formulations, which include suspensions, puffs from inhalers, viscous slurry, or orodispersible tablets. Fluticasone given orally in the form of a spray from a metered-dose inhaler and liquid budesonide as a viscous preparation are the typical pharmacologic therapies used for EoE[32,33]. Fluticasone (440-880 μg twice daily) or budesonide (1-2 mg twice daily) for 8 wk can be used to induce remission[34]. In 2020, the European Medicines Agency approved an orodispersible budesonide tablet formulation for adults with EoE[21,22].

Long-term topical glucocorticoid therapy is indicated in EoE due to frequent recurrence with tetracycline antibiotics removal. Maintenance therapy with topical steroids and dietary restriction should be considered, especially in patients with severe symptoms (*i.e.*, dysphagia, food impaction, and weight loss), anatomical complications (high-grade esophageal strictures), and rapid relapse after initial therapy[19]. Clinicians must pay close attention to side effects, such as esophageal candidiasis.

Esophageal dilatation

This treatment is reserved for patients with EoE that do not benefit from conservative therapy or for patients with EoE that show high-grade strictures. Endoscopic dilation can provide immediate symptomatology improvement in 95% of patients with EoE that have narrow caliber esophagus or strictures [34]. Since dilation has only a mechanical effect and does not stop the inflammation of the underlying eosinophil, repeated treatments are normally required to keep symptoms under control. The association of medical therapy is recommended in these individuals[34].

Biologic agents

Current evidence suggests that type 2 cytokines play a key role in EoE[25]. Monoclonal antibodies are used in other Th2-mediated allergic diseases and have the potential of modifying the natural history of the disease. Treatments that block the underlying inflammatory processes and prevent disease progression can be useful.

IL-13 has been implicated as an important cytokine in the pathogenesis of EoE. *The New England Journal of Medicine* has recently published the results of a three-part, randomized, double-blind, placebo-controlled trial based on dupilumab, a fully human monoclonal antibody that blocks IL-13 and IL-4 signaling[35]. Eligible patients were aged 12 years or older and had a diagnosis of EoE by endoscopic biopsy despite 8 wk of high-dose PPI therapy. The two primary endpoints after 24 wk included histologic remission (≤ 6 eosinophils using a high-power field) and the decrease from baseline in the Dysphagia Symptom Questionnaire score. The study demonstrated that dupilumab at a weekly dose of 300 mg led to reductions in symptoms of EoE and enhancements in histologic outcomes amongst adolescents and adults[35]. The most frequently reported adverse event throughout the period of treatment with dupilumab was an injection-site reaction. Other common adverse reactions were respiratory tract infections, arthralgia, and herpes viral infections. Dupixent (dupilumab) was approved by the Food and Drug Administration in May of 2022 for the treatment of EoE in patients 12 years and

older weighing at least 40 kg[36].

Another biological agent that has been considered in the treatment of EoE is mepolizumab. This agent is a humanized monoclonal antibody against IL-5, a cytokine that is crucial for the recruitment of eosinophils. Studies regarding mepolizumab, however, have shown variable results[37,38]. Other biologics such as cendakimab (a monoclonal antibody inhibiting IL-13 receptor binding), lirentelimab (a monoclonal antibody to sialic acid-binding immunoglobulin-like lectin 8, a CD33 receptor present on the surface of mast cells and eosinophils), and bevacizumab (a monoclonal antibody directed against the membrane-bound IL-5 receptor α chain present on eosinophils) are in various stages of clinical trials [39]. Some of these drugs have been approved for other atopic conditions, and thus appear as potentially promising treatment options for EoE and other eosinophilic gastrointestinal diseases. Further studies are needed to determine the long-term treatment outcomes for each of these drugs[39].

CONCLUSION

EoE remains to be one of the most frequent eosinophilic gastrointestinal diseases. Standard treatments with diet modifications, PPIs, and topical corticosteroid preparations have variable rates of response. Current studies in the literature have been enriching the understanding of the mechanisms and pathogenesis involved in EoE disease, which can help find potential disease-modifying biologic therapies, such as dupilumab, as an effective treatment option. Further studies are needed to determine the long-term outcomes of these drugs. The use of these agents in EoE offers a potentially promising option for patients with severe symptoms and complications.

FOOTNOTES

Author contributions: Grando M, De Pauli S, and Zeppieri M wrote the outline and did the research and writing of the manuscript; Grando M, De Pauli S, Miotti G, Balbi M, and Zeppieri M assisted in the writing of the paper; Zeppieri M (a native English speaker, MD, PhD) was responsible for the conception and design of the study and completed the English and scientific editing; Grando M, De Pauli S, and Zeppieri M assisted in the editing and making critical revisions of the manuscript; and all authors provided the final approval of the article.

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Country/Territory of origin: Italy

ORCID number: Martina Grando 0000-0002-1877-3621; Silvia De Pauli 0000-0002-0486-0441; Giovanni Miotti 0000-0003-3185-7595; Massimiliano Balbi 0000-0002-4757-1009; Marco Zeppieri 0000-0003-0999-5545.

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MINIREVIEWS

IgA nephropathy associated with Crohn's disease

Hiroshi Tamura

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Hiroshi Tamura, Department of Pediatrics, Kumamoto University, Kumamoto 8608556, Japan

Corresponding author: Hiroshi Tamura, MD, PhD, Assistant Professor, Department of Pediatrics, Kumamoto University, Chuo-ku Honjo 1-1-1, Kumamoto 8608556, Japan.
bohm1905ht@kuh.kumamoto-u.ac.jp

Abstract

The relationship between IgA nephropathy (IgAN) and Crohn's disease was reported. IgAN is the most common primary glomerulonephritis and one of the leading causes of chronic kidney disease and end-stage renal failure, and up to 50% of cases progressed to end-stage renal disease within 25 years after IgAN diagnosis. However, specific and effective therapeutic strategies are still lacking. In this review, we discuss the possibility of the mechanism involved in IgAN associated with Crohn's disease based on the findings of basic and clinical studies. Although the etiology of IgAN associated with Crohn's disease is not permanent and various factors are thought to be involved, the stabilization of the disease condition of Crohn's disease is believed to help treat IgAN.

Key Words: Crohn's disease; IgA nephropathy; Immunological abnormalities; Mucosal-associated lymphoid tissue; Gut-associated lymphoid tissue; Nasopharynx-associated lymphoid tissue

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Core Tip: The relationship between IgA nephropathy (IgAN) and Crohn's disease was reported. Crohn's disease (CD) immunological abnormalities may promote and activate the IgAN inflammatory process. Although the etiology of CD-IgAN is not fixed and various factors are thought to be involved, the stabilization of the disease condition of CD is believed to help treat IgAN.

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INTRODUCTION

Intestinal diseases include chronic nonspecific inflammatory diseases of the gastrointestinal tract. Inflammatory bowel disease (IBD) is a chronic and recurrent inflammatory disease of the gastrointestinal tract that includes crohn's disease (CD) and ulcerative colitis (UC).

In addition to intestinal inflammation, CD has numerous extraintestinal manifestations of IBD. Extraintestinal complications of renal and urological diseases in CD include internal fistula lesions such as enterovesical fistula and enterovaginal fistula, urolithiasis, and renal disease. Urolithiasis is relatively common in 19% of patients with IBD; however, comorbid renal disease is relatively rare. Interstitial nephritis and secondary renal amyloidosis caused by 5-aminosalicylic acid (5-ASA) are renal diseases that show a clear causal relationship with CD treatment or CD itself; however, glomerulonephritis has been reported in few cases, and the lack of a causal relationship is difficult to prove. Among them, the relationship between IgA nephropathy (IgAN) and CD was reported[1,2].

IgAN is the most common primary glomerulonephritis and one of the leading causes of chronic kidney disease and end-stage renal failure. Up to 50% of cases progressed to end-stage renal disease (ESRD) within 25 years after the diagnosis of IgAN. However, at present, specific and effective therapeutic strategies are still lacking[3,4]. Thus, this paper discusses the possibility of the mechanism involved in IgAN associated with CD (CD-IgAN) based on the findings of basic and clinical studies.

ABNORMAL MUCOSAL IMMUNITY AND IGAN

Because IgAN exacerbates nephropathy after upper respiratory tract infection, it has been hypothesized that mucosal immunity is involved in the pathogenesis of IgAN. IgA is an immunoglobulin that mainly acts on the mucosa, and renal glomerular IgA is a mucosal multimeric IgA1 containing a secretory component[5-8], and multimeric IgA1 is also increased in the serum[9,10]. These have also supported these hypotheses.

Conversely, which mucosal-associated lymphoid tissue (MALT) is the main responsible site for IgAN is unclear. The involvement of nasopharynx-associated lymphoid tissue (NALT) is assumed to be mainly responsible for IgAN because it worsens after upper respiratory inflammation. Gut-associated lymphoid tissue (GALT) has a huge mucosal area and is the main production site of mucosal IgA; however, its involvement in the pathology of IgAN is controversial. In addition, the exacerbation of IgAN after a mucosal infection suggests the involvement of foreign antigens; however, many points are unclear about how infection is involved.

Recently, the activation of the innate immune system triggered by infection is involved in the onset and progression of various types of nephritis, along with mechanisms such as molecular mimicry and epitope spreading[11]. However, the specific mechanism of IgAN has not been elucidated.

IgAN leads to ESRD, IgAN recurrence in approximately half of the patients who underwent kidney transplantation, and vice versa. IgAN disappears when the kidney is transplanted as a donor, suggesting that the main cause of IgAN is not kidney-specific cells but the systemic IgA immune system [5].

The facts suggesting the involvement of mucosal immunity are as described above; however, bone marrow abnormalities have also been pointed out in patients with IgAN. van den Wall Bake *et al*[12] reported that the ratio of IgA-producing plasma cells (IgA + PC) increased in the bone marrow of patients with IgAN, and IgA1 production was enhanced. These suggest that glomerular IgA1 is derived from the bone marrow[13].

Moreover, in patients with IgAN, IgA1 was significantly higher with the production of peripheral blood mononuclear cell IgA[12]. By contrast, Harper *et al*[14] reported that J-chain mRNA-positive mucosal-type IgA + PC increased in the bone marrow of patients with IgAN. Another study reported that when leukemia develops in a patient with IgAN and the patient undergoes bone marrow transplantation, not only leukemia but also IgAN improves[15]. In other words, in patients with IgAN, mucosal-type IgA + PC, which is involved in the pathology, increased in the extramucosal bone marrow, suggesting the excessive production of mucosal-type multimeric IgA1[5].

Approximately 30 years ago, van den Wall Bake *et al*[16] hypothesized the existence of "mucosa–bone marrow axis" abnormalities in IgAN[5]. Moreover, the homing mechanism of immunocompetent cells to the effective tissue was clarified. This hypothesis became more realistic because mucosa-derived effector cells were found to be translocated and stored in the lymphatic tissue.

Studies using mouse models of spontaneous IgAN have also shown that cells responsible for the abnormal IgA production related to renal onset are present in the bone marrow and spleen[17-19]. Active cell migration and immune information exchange take place between the mucosal tissue and bone marrow. However, whether the cells responsible for IgAN migrate between the MALT and bone marrow or whether only relatively localized mucosal tissue is involved is unclear.

Moreover, we discuss the possible involvement of GALT and NALT in pathologies with IgAN. Serum IgA is mainly produced in the bone marrow, and intestinal mucosa-derived IgA is thought to be scarce. Conversely, in GALT, large amounts of IgA are produced and secreted in the mucosa, which contribute

to intestinal immunity. Unlike mucosal IgA, the physiological role of serum IgA is largely unknown. Although there are IgA1 and IgA2 subclasses in humans, 80%-85% of serum IgA is IgA1[20].

As shown in the IgA1 and IgA2 ratios of IgA + PC in the mucosal execution phase, IgA2 + PC was high (30%-65%) in the intestinal mucosa, whereas it was low (7%-25%) in the peripheral lymph nodes and respiratory tract mucosa[20]. In particular, IgA2 + PC is dominant in the ileum and colon[21-24].

Human IgA2 Lacks amino acids, which are the recognition sites of hinge-specific proteases derived from intestinal bacteria, and is protease resistant, which is thought to work favorably in intestinal immunity[24]. The organogenesis mechanisms of Peyer's patch (PP) and NALT are significantly different.

In the PP, the number of $CD3^{-}CD4^{+}CD45^{+}$ inducer cells increased from the embryonic period and decreased until 3 wk after birth. In NALT, the number of inducer cells increased because of postnatal stimulation with foreign antigens and peaks at 3 wk after birth[25]. The molecular mechanisms involved in the organogenesis of the two are also different[26]. These facts suggest the difference in the basic roles and needs of both in the mucosa.

The polymeric immunoglobulin receptor (pIgR) is a protein expressed on the basolateral side of mucosal secretory epithelial cells that carry IgA and IgM produced in the mucosa to the mucosal surface.

In GALT, IgA and IgM produced from the PC of the lamina propria (LP) form dimeric IgA and pentameric IgM by the J-chain produced at the same time. pIgR easily binds to multimeric IgA and IgM containing this J-chain and efficiently transports them to the mucosal surface. The transported multimeric IgA and IgM trap antigens in the mucosa and act as a non-inflammatory mucosal immune defense mechanism[27,28].

In IBD, IgA is actively produced in the LP. Conversely, inflammatory pIgR expression and dysfunction occur, inhibiting its secretion on the mucosal surface. Therefore, mucosal IgA, which increased in the LP, was also thought to increase in the blood[29]. Mucosal IgA in the blood is increased in patients with IBD such as UC and CD[30].

The lymphotoxin-β receptor (LTβR) is essential for the formation of mucosal lymphoid tissue[31] and plays an important role in IgA production in the small intestine[32].

LIGHT is a ligand for LTβR and is expressed on activated T cells[33]. In LIGHT transgenic mice (LIGHT Tg), sustained stimulation of LIGHT *via* LTβR on T cells causes the over-induction of IgA + PC in the LP, and increased expression of MAdCAM-1 by stimulation from LTβR leads to the activation of inflammatory cells, causing severe enteritis[30].

Impaired pIgR expression and excessive IgA production caused by this intestinal inflammation induce mucosal multimeric IgA, which is not transported to the mucosa, to enter and increase their presence in the blood circulation. Because these multimeric IgAs have high affinity to the kidney, they are deposited in the kidney and cause IgAN[30].

On the contrary, the bias of Th2 cytokines may be involved in the glycosylation of IgA in the intestinal tract. Even a small amount of IgA released into the blood from the intestinal tract may cause nephropathy[34-36]. Thus, induction of glomerular deposition of intestinal IgAN is theoretically possible if conditions such as inflammation-associated pIgR dysfunction are met.

However, clinically, most patients with IgAN do not have IBD or gastrointestinal symptoms, and the so-called secondary IgAN associated with intestinal abnormalities occurs in a small proportion of patients. The involvement of IBD in IgAN is considered limited, at least in terms of IgAN in Japan.

COMPARISON OF HISTOLOGICAL FINDINGS BETWEEN CD□IGAN AND COMMON IGAN

Virchows Archiv investigated the clinical and pathological differences CD-IgAN and common IgANs (NOS-IgAN) associated with upper airway inflammation such as tonsillitis[37]. The PCs of the upper airway mucosa mainly produce IgA1[38]. However, the intestinal mucosa, especially PP, is thought to predominantly secrete IgA2 (approximately 60% in mucosal cells) rather than IgA1[39]. For IgAN with CD, intestinal IgA2 is deposited in the glomerular mesangium and may be involved in IgAN induction and progression. However, among the deposited IgA subclasses, the IgA1 subclass was predominant in both the CD-IgAN and NOS-IgAN groups, and no significant difference in the staining intensity of IgA2 was found between the two groups. Then, they examined the deposition of galactose-deficient IgA1 (Gd-IgA1) in the glomerulus of primary IgAN[40].

Gd-IgA1 is an abnormal IgA1 of the IgA1 subclass, exhibiting a structure in which the o-linked glycans in the hinge lack galactose and expose N-acetylgalactosamine (GalNAC)[41,42]. They found no difference in the extent of Gd-IgA1 deposition in the glomeruli, irrespective of CD complications, and a significant difference in Gd-IgA1 deposition was found the between CD-IgANs and NOS-IgAN. CD-IgANs were suggested to share the same pathogenesis as primary IgANs. Furthermore, negative views have emerged regarding the disease specificity of Gd-IgA1[43]. In their renal histological findings, patients with CD-IgAN were found to have significantly more severe glomerulosclerosis, arteriolar hyalinosis grade, tubular atrophy, and interstitial fibrosis than those with NOS-IgAN.

A comparison of the Oxford Classification MEST-C scores revealed that the T-scores representing tubular atrophy and interstitial fibrosis were significantly higher in CD-IgANs than in NOS-IgANs. To confirm this trend, they performed a meta-analysis comparing MEST-C scores using a large cohort of patients with IgAN reported in previous studies[44,45]. The incidence of T1 and T2 was higher in CD-IgANs than in IgANs. These histological differences were speculated to be related to the following factors: (1) CD pathophysiology (*i.e.*, diarrhea and dehydration); (2) Therapeutic agents for CD; and (3) Systemic inflammation, including the gut.

First, in the course of CD, dehydration due to diarrhea, reduced fluid intake, and surgery can reduce the circulating blood volume, which can lead to tubulointerstitial damage and glomerulosclerosis. In addition, undernutrition and hypokalemia have been reported to cause chronic tubulointerstitial injury [46]. The possible reason is that the reduced effective circulating blood volume stimulates the renin–angiotensin–aldosterone system, which in turn enhances the activation of angiotensin II, which can lead to arteriolar contraction, glomerular ischemia, and interstitial fibrosis[47,48]. In addition, evidence showed that hyperuricemia, common in CD, may exacerbate glomerulosclerosis[49].

Second, this factor is related to the effect of treatment. 5-ASA remains the main treatment for CD, and its adverse renal effects are collectively called mesalamine-associated kidney disease[50]. The mechanism by which mesalamine promotes renal injury appears to be through the salicylic acid inhibition of intrarenal prostaglandin synthesis, which is a vasoactive mediator of intrarenal blood flow and uncouples mitochondrial oxidative phosphorylation[46,51].

Several studies have also reported that mesalamine promotes histologically renal injury through interstitial nephritis[52], and similar findings have been reported in patients with CD, not on 5-ASA [53]. Therefore, distinguishing whether interstitial nephritis is due to drugs or CD is challenging.

Third, in CD pathogenesis, immune abnormalities may be involved. Macrophages and T cells produce large amounts of interleukin-23 and tumor necrosis factor (TNF)-α in immune diseases such as CD and are thought to play a central role in CD pathophysiology[54]. These cytokines also contribute to the exacerbation of IgAN tubulointerstitial lesions[54]. A study has also reported a mechanism by which dysfunctional macrophages promote intestinal fibrosis[55].

In recent years, the mechanisms and systemic responses of B cell immune abnormalities in CD have been elucidated[56-58]. Interstitial inflammation in chronic kidney disease involving IgAN was reported to involve B cell-mediated immune dysfunction[59], and immune dysfunction in CD was found to be associated with IgAN in terms of immune dysfunction, which may act as an aggravating factor for renal function. Moreover, in IgAN, complement activity was thought to promote glomerulosclerosis and interstitial fibrosis[60,61].

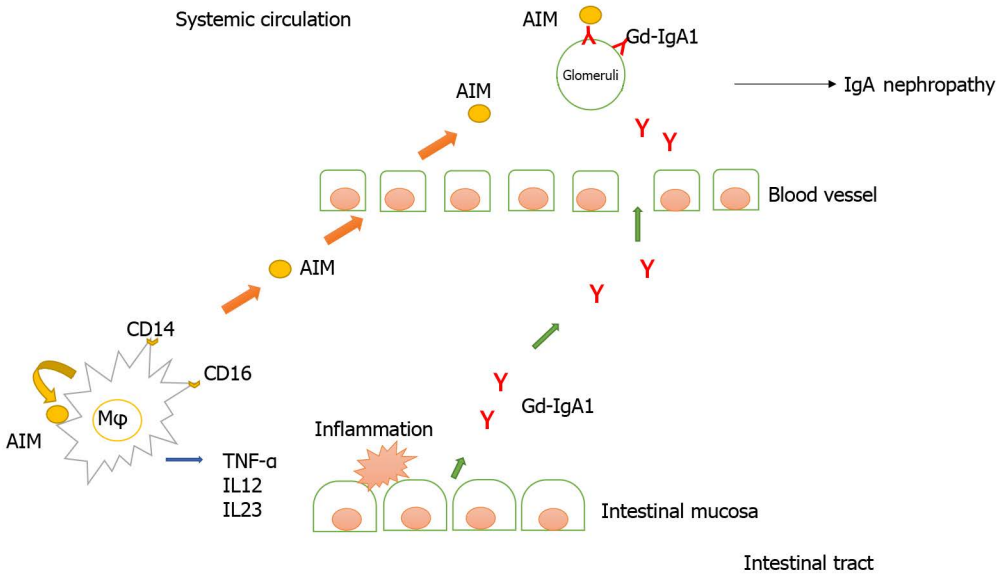
Previous studies have shown that in patients with CD, activated complement (predominantly C3b) stains strongly in the intestinal mucosa[62] and that resected ileocecal specimens show increased expression of complement C3 mRNA[63]. The effects of CD-associated complement activation may influence glomerular and tubulointerstitial inflammations in IgAN.

COMMON KEY GENES ASSOCIATED WITH CD AND IGAN

Several common genetic predispositions to CD and IgAN have been reported. *HLA-DR1* in IgAN[64] and *HLA-DR1 DQw5* in CD[65] are characteristic genetic predispositions. Moreover, *HLA-DR1*-positive patients may have an increased risk of IgAN and CD[66]. A genome-wide association study found that *CARD9*, *HORMAD2*, and *HLA-DQB1*susceptibility genes for IgAN were also associated with IBD. These IgAN loci encode a protein involved in maintaining the intestinal barrier and regulating mucosal immune responses to pathogens[67-70]. Yuan *et al*[71] reported that the gene for CXCL2 is critically linked to immune infiltration during CD and IgAN. CXCL2, also known as macrophage inflammatory protein-2, belongs to the CXC subfamily. It is secreted by many cell types, including monocytes, macrophages, endothelial cells, and hepatocytes, in response to infection and injury. It primarily affects the recruitment of polymorphonuclear leukocytes to sites of injury or infection, thereby modulating immune and inflammatory responses. The analysis of the relationship between CXCL2 and immune cell infiltration in CD and IgAN diseases suggested that CXCL2 is involved in immune infiltration, thereby contributing to the pathogenesis of the two diseases.

PATHOGENESIS OF IGAN ASSOCIATED WITH CROHN'S DISEASE

Recent research has revealed that the apoptosis inhibitor of macrophages (AIM) is involved in the pathogenesis of renal failure through the function of macrophages, a type of leukocytes. AIM deposited in the same sites as IgA in the glomeruli in human IgAN and a spontaneous mouse model of IgAN. As a result, AIM deficiency in the spontaneous IgAN mouse model, although IgA deposition in the glomeruli was observed, did not develop IgAN. Subsequently, when AIM was administered to mice, IgG and IgM were co-deposited in the glomeruli, proteinuria and occult blood appeared, and nephropathy was confirmed. From this, IgA deposition in the glomerulus alone does not progress to nephropathy. In



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Figure 1 Intestinal mucosal barrier disruption due to Crohn's disease enhances the production of glycosylated IgA. Apoptosis inhibitor of macrophages (AIM) produced by resident macrophages in the intestinal tissue contributes to intestinal inflammation, and active macrophage-derived AIM in the gut leads to elevated serum AIM levels. AIM is involved in the recruitment of inflammatory macrophages *via* autocrine and paracrine and induces inflammatory cytokine (TNF-α) in mesenteric adipose tissue in Crohn's disease. AIM influences the pathogenesis of Crohn's disease by inhibiting apoptosis of active intestinal macrophages and enhancing the expression of TNF-α in these mesenteric adipose tissues. Intestinal mucosal barrier disruption due to Crohn's disease enhances the production of glycosylated IgA. In IgA nephropathy, it was clarified that the deposition of IgA in the glomerulus is essential as the first step, and that AIM leads to inflammation as the second step. AIM: Apoptosis inhibitor of macrophages; CD: Crohn's disease; Mφ: Macrophages.

addition, glomerular deposition of AIM, IgG, and IgM was not induced in IgA-deficient mice. These results demonstrate that IgAN involves the deposition of IgA into the glomeruli as the first step and that co-deposition of IgG and IgM *via* AIM leads to inflammation as the second step. Furthermore, in human IgAN, AIM was co-deposited with glomerular IgA, IgG, IgM, and complement (C3). Therefore, AIM is a key molecule that initiates inflammation in IgAN[72].

Ono *et al*[73] showed that serum AIM levels were higher in patients with CD than in patients with UC, patients with intestinal BD, and healthy controls. Furthermore, AIM is expressed in CD14- and CD16-positive macrophages in the intestinal tissue.

AIM produced by resident macrophages in the intestinal tissue was believed to contribute to intestinal inflammation and that active macrophage-derived AIM in the intestine results in elevated serum AIM levels. AIM is taken up by the adipocytes *via* CD36-mediated endocytosis, which subsequently induces lipolysis[74,75]. Therefore, it may be involved in the recruitment of inflammatory macrophages and induce TNF-α in mesenteric adipose tissue in CD.

AIM may affect the pathogenesis of CD by not only inhibiting the apoptosis of active intestinal macrophages but also enhancing TNF-α expression in these mesenteric adipose tissues.

Inoue *et al*[76] reported that glycosylated IgA is produced in the gastrointestinal mucosa of CD and that glycosylated IgA correlates with CD disease activity. These results are crucial because they not only indicate that CD induced IgAN but also serve as evidence of the correlation between CD and IgAN.

We hypothesized the pathogenesis of IgAN associated with Crohn's disease (Figure 1).

IGAN: CD-ASSOCIATED OR ADALIMUMAB-INDUCED?

We searched PubMed for studies on CD with IgAN (Supplementary Figure 1). Twenty-five cases have been reported[77-92], including a self-case, with an average age of 27.5 (9–39) years, male-to-female ratio of 20:5 (male 80%), seven cases with underlying IgAN, and seven cases with underlying CD. Moreover, 17 patients had comorbidities, and one case had a simultaneous onset (Table 1).

However, certain cases had urinalysis abnormalities such as proteinuria and microscopic hematuria, which have been pointed out before the definitive diagnosis of IgAN. Because CD also progresses preclinically, identifying the time of onset of CD and IgAN is very difficult.

In the literature, the pathological findings of CD-complicated nephritis and IgAN with CD also tended to have a higher T-score, which represents tubular atrophy and interstitial fibrosis, than IgAN without CD. Five patients developed IgAN during biologic therapy, and CD and proteinuria improved after biologics were discontinued or changed.

Table 1 Reported cases of Crohn's disease with IgA nephropathy

Age (yr)	Sex	Clinical course	CD disease type	IgAN MEST-C grade	CD treatment	IgAN treatment	CD and IgAN worsened simultaneously
20	Female	CD(y)→IgAN(y)	Ileocolitis	NR	SASP	nothing	+
25	Male	CD(4y)→IgAN(12y)	Ileocolitis	NR	ED only	NR	+
18	Male	IgAN(NR)→CD(2y)	colitis	NR	5-ASA	ACE-I	NR
24	Male	CD(NR)→IgAN(3y)	NR	NR	5-ASA	NR	-
38	Male	CD(NR)→IgAN(6y)	NR	NR	PSL+5-ASA →IFX	ACE-I	+
40	Female	CD(NR)→IgAN(5y)	colitis	NR	5-ASA	ARB	+
38	Male	CD(NR)→IgAN(16y)	Ileocolitis	NR	5-ASA	NR	NR
40	Male	CD(NR)→IgAN(12y)	NR	NR	PSL+5-ASA	PSL	+
34	Female	IgAN(4y)→CD(5y)	Ileocolitis	NR	ED only	PSL	+
25	Male	CD(25y)→IgAN(33y)	NR	M1E1S1T1C1	ADA	MPT→PSL, ACE-I	-
24	Male	CD(22y)→IgAN(24y)	Ileitis	M1E0S1T1C2	IFX →UST	MPT→PSL	-
10	Male	CD(1y)→IgAN(7y)	colitis	M1E0S0T0C0	IFX	IFX→ADA, PSL	+
40	Male	CD(NR)→IgAN(39y)	NR	M1E0S1T0C1	AZA	PSL	-
31	Male	CD(16)→IgAN(18y)	Ileocolitis	M1E0S1T1C0	PSL+5-ASA	PSL	+
46	Male	IgAN(17y)→CD(40y)→IgAN(46y)	NR	M1E0S0T0C1	5-ASA, AZA, IFX	PSL	+
15	Female	CD(16)→IgAN(18y)	NR	NR	5-ASA, PSL	IFX	+
13	Male	IgAN(10y)→CD(13y)	NR	M1E0S0T0C1	PSL, SASP	PSL, CPA, ACE-I	+
9	Male	CD(9)→IgAN(11y)	Ileitis	M1E0S0T1C0	ADA	ADA →AZA; MPT, PSL, ACE-I	-
39	Female	CD(32)→IgAN(39y)	NR	M1E0S1T0C0	ADA, AZA	ARB, ADA→IFX	+
27	Male	IgAN(22y)→CD(27y)	NR	M1E0S1T2C1	5-ASA, IFX	MPT, MMF, CsA	+
18	Male	CD(9)→IgAN(11y)	NR	M1E1S1T1C2	5-ASA	PSL, CPA	+
22	Male	IgAN(8y)→CD(22y)	NR	M1E1S1T1C0	SASP, AZA	PSL, 5-ASA	+
36	Male	CD(30)→IgAN(36y)	NR	M1E0S0T2C0	5-ASA, AZA	ACE-I	-
29	Male	IgAN(24y)→CD(29y)	colitis	NR	PSL	non	+
10	Male	CD(30)→IgAN(36y)	Ileocolitis	M1E0S0T0C0	ADA, AZA, budesonide	PSL	+

CD: Crohn's disease; IgAN: IgA nephropathy; ED: Elemental diet; PSL: Prednisolone; MPT: Methylprednisolone pulse therapy; SASP: Salazosulfapyridine; 5-ASA: 5-aminosalicylic acid; CPA: Cycrophosphamid; AZA: Azathioprine; IFX: Infliximab; ADA: Adalimumab; UST: Usutekinumab; CsA: Cyclosporin; MMF: Mycophenolate mofetil; ACE-I: Angiotensin converting enzyme inhibitor; ARB: Angiotensin receptor blocker; NR: Not reported.

Simultaneous exacerbation of both diseases was observed in 17 of 23 patients, and many studies have reported that the disease activity of both diseases was related, suggesting that IgAN is an extraintestinal complication of CD. This tendency was also observed in our case (Figure 2). Considering that the disease progression of both diseases is the same, controlling the disease progression of CD is also important to prevent the progression of renal failure.

The use of immunomodulators to maintain remission in both diseases is an option. In addition, because TNF-α induces glomerular inflammation and enhances permeability[93], anti-TNF-α antibodies are theoretically expected to improve proteinuria.

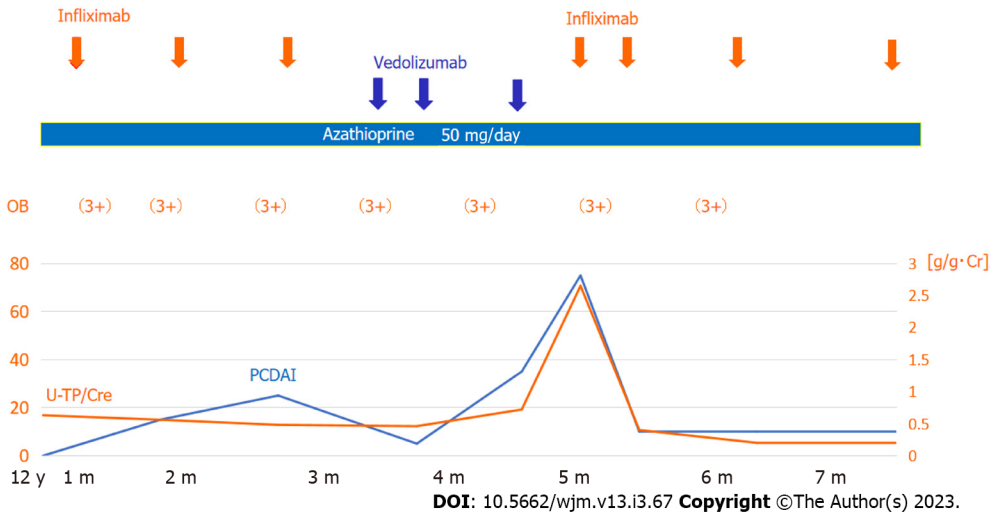


Figure 2 Clinical course after the appearance of hematuria. Hematuria and proteinuria persisted, and IgA nephropathy was suspected. Since there were some reports of IgA nephropathy caused by infliximab, we switched from infliximab to vedolizumab. After the change to vedolizumab, Crohn's disease worsened, and there was a marked increase in proteinuria. Fever and abdominal symptoms worsened, and infliximab was administered again. Thereafter, Crohn's disease improved rapidly, and decrease in the urinary protein level was noted. OB: Occult blood urine; U-TP/Cre: Urinary protein creatinine ratio; PCDAI: Pediatric Crohn's disease activity index; y: years; m: Months.

However, TNF-α inhibitors were also reported to induce systemic vasculitis and several types of glomerulonephritis, such as minimal-change disease group, membranous nephropathy, IgAN, and lupus nephritis[94-98].

Although the following hypotheses have been suggested and the following possibilities have been proposed, the mechanism of vasculitis associated with anti-TNFα therapy is unclear: (1) TNFα/anti-TNF α immune complexes may deposit in small blood vessels and induce local complementary activation [94]; (2) A cytokine imbalance with a shift from a Th1 profile to a Th2 profile could induce symptoms associated with antibody-mediated immunity[95]; and (3) In glomerulonephritis, immune complexes are thought to form by cross-reactivity of Gd-IgA1 and anti-drug antibodies to the glycan structures of TNFα inhibitors. They are deposited in the mesangium and can induce IgAN[96].

The reported detection rate of antinuclear antibodies in patients treated with anti-TNFα ranged from 29% to 76.7%. Immunological abnormalities can lead to glomerulonephritis, including membranous glomerulonephritis and lupus nephritis[97,98].

CONCLUSION

In many cases, proteinuria progressed simultaneously with CD; moreover, it is possible that IgANand Crohn's disease occur in parallel. The factors governing the simultaneous occurrence of IgAN and Crohn's disease is still unknown; however, the involvement of biologics has been pointed out. Although the etiology of CD-IgAN is not fixed and various factors are thought to be involved, the stabilization of the disease condition of CD is believed to help treat IgAN.

FOOTNOTES

Author contributions: Tamura H reviewed the patient's clinical data, wrote the initial draft of the manuscript, contributed to writing the manuscript, and revised the final version of the manuscript.

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Country/Territory of origin: Japan

ORCID number: Hiroshi Tamura 0000-0001-8786-217X.

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Wattana Leowattana, Pathomthep Leowattana, Tawithep Leowattana

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Wattana Leowattana, Pathomthep Leowattana, Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Rachatawee 10400, Bangkok, Thailand

Tawithep Leowattana, Department of Medicine, Faculty of Medicine, Srinakharinwirot University, Wattana 10110, Bangkok, Thailand

Corresponding author: Wattana Leowattana, BMed, MD, MSc, PhD, Full Professor, Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajavithi road, Tungphyathai, Rachatawee 10400, Bangkok, Thailand.
wattana.leo@mahidol.ac.th

Abstract

Gastric cancer (GC) is believed to be the fifth most common cancer and the third most common cause of death worldwide. Treatment techniques include radiation, chemotherapy, gastrectomy, and targeted treatments are often employed. Some hopeful results from the development of GC immunotherapy have already changed treatment approaches. Along with previous combination medicines, new immunotherapies have been developed that target distinct molecules. Despite ongoing studies into the current therapeutic options and significant improvements in this field, the prognosis for the ailment is poor. Since there are few treatment options and a delay in detection, the illness actually advances, spreads, and metastasizes. The bulk of immunotherapies in use today rely on cytotoxic immune cells, monoclonal antibodies, and gene-transferred vaccines. Immune checkpoint inhibitors have become more popular. In this review, we sought to examine the viewpoint and development of several immunotherapy treatment modalities for advanced GC, as well as the clinical results thus far reported. Additionally, we outlined tumor immune escape and tumor immunosurveillance.

Key Words: Immunotherapy; Advanced gastric cancer; Personalized medicine; Biomarkers; Chemotherapy; Cancer vaccine

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Core Tip: Throughout the globe, gastric cancer (GC) is ranked as the fifth most frequent cancer and the third most common cause of death. Chemotherapy, radiation, stomach resection, and targeted treatments are common treatment modalities. The development of immunotherapy for GC has already produced some encouraging outcomes and changed the treatment process. Currently, new immunotherapies that target novel molecules, as well as other combination treatments, have been developed. Immune checkpoint inhibitors are being used more and more often. In this review, we sought to examine the viewpoint, development, and reported clinical results of several immunotherapy treatment modalities for advanced GC patients.

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INTRODUCTION

The third leading cause of cancer-related mortality is gastric cancer (GC), which includes adenocarcinomas of the gastroesophageal junction (GEJ) and stomach. GC is the fifth most frequent cancer around the globe. Eastern Europe, Eastern Asia, and South America have the highest prevalence rates of GC. The majority of patients in Western nations receive their diagnoses at an advanced stage, which is defined by metastatic dissemination that is inoperable[1-3]. Long-term disease control has not yet been accomplished, despite the emergence of novel treatments. As a result, advanced GC (AGC) has a terrible prognosis, with a 5-year survival rate of only 10%. While cardiac GC is more common in North America, Australia, and the United Kingdom, non-cardiac GC is more common in Eastern Asia. The most well-known cause of GC is *Helicobacter pylori* (*H. pylori*) infection, while Epstein-Barr virus (EBV) infection has also been associated with the development of GC. Cigarette smoking, obesity, a high salt intake, a poor intake of fruits and vegetables, and a high intake of salted preserved foods are some lifestyle choices that have been linked to an increased risk of GC[4,5].

The prognosis for AGC is still bleak despite recent improvements in multimodal therapy. The GC's extremely complicated molecular basis is one of the factors contributing to its dismal prognosis. Numerous genetic and epigenetic changes, including differential gene expression, gene mutations, DNA/histone methylation, and somatic copy number changes, have been shown to contribute to the aggressive phenotype of GC. No encouraging and treatable causes of GC have yet been discovered, regardless of the fact that it is a diverse disease that is probably caused by several genetic and epigenetic abnormalities[6-8]. Although no successful therapies based on molecular characterization have been created yet, the creation of more effective treatment strategies based on novel molecular data may be feasible in the future. Nowadays, the median survival time with the best supportive care varies from 4 mo to 12 mo with standard cytotoxic treatment. Throughout the last several decades, advances in knowledge of cancer's molecular etiology and biology have resulted in the creation of innovative targeted therapy techniques that have led to higher survival rate in some contexts. These targeted therapies are also offered as small molecule inhibitors and monoclonal antibodies (mAbs), most of which are tyrosine kinase inhibitors (TKIs). Therefore, current systemic therapies for metastatic GC combine cytotoxic chemotherapy with first- and second-line therapies using targeted medicines such as trastuzumab and ramucirumab, respectively. Additionally, the establishment of immune checkpoint inhibition in the past ten years has been recognized as a significant medical and scientific advancement in the battle against malignancy; however, studies looking at the use of immunotherapy in GCs, either as a single agent or in conjunction with cytotoxic chemotherapy, have only produced limited authorization in the second-line setting, after the failure of the initial treatment, with comparably modest rates of response ranging between 5% and 30%[9-11]. The goal of this review is to quickly highlight some of the most promising immunotherapies now being researched while also summarizing the currently investigated and authorized treatments for GC.

EPIDEMIOLOGY

GC is often asymptomatic in the early stages, making it challenging to purposefully discover. Late diagnosis is mostly to account for the high mortality of GC. In order to lower GC mortality, early identification and treatment are essential[12,13]. Some East Asian nations with high relative risks have implemented their own extensive screening programs. Regardless of whether an individual has symptoms, upper gastrointestinal endoscopy is available in these nations. Endoscopic screening can lower GC mortality by 67% compared to radiography screening, according to Japanese population-

based cohort research[14]. Endoscopy was the most economical screening technique, according to data from the National Cancer Screening Program in South Korea, which may enhance survival rates[15]. In addition, the quality of endoscopic imaging has recently dramatically improved. Endoscopy using image enhancement techniques, including narrow-band imaging, can help with early GC discovery and complete endoscopic resection. Indeed, these active screening methods have resulted in earlier discovery and a higher survival rate[16,17]. The 5-year relative survival rate in Japan between 2009 and 2011 was reported at 66.6%, with more than 60% of cases of GC being discovered at stage I, according to population-based statistics collected countrywide[18]. In contrast to Asian nations, Western nations lack widespread screening programs, which causes discovery to occur later. According to the SEER-based CONCORD-2 research in the United States, only 22.1% (2001-2003) or 24.9% (2004-2009) of patients had a localized stage at diagnosis, and in comparison to Asian countries, the stated 5-year survival rate was lower (26.1% from 2001-2003 and 29.0% from 2004-2009)[19]. Western nations have a higher prevalence of GC in the proximal third. Proximal GCs are more likely to be in an advanced stage at presentation, be larger, and have a histology that is poorly differentiated. The poorer survivability in the West may be explained by this[20,21].

In particular, for intestinal-type distal carcinoma, *H. pylori* infection raises cancer risk. In comparison to Europe (47.0%) and North America (37.1%), Asia has a greater prevalence of *H. pylori* (54.7%). It is well known that the elimination of *H. pylori* causes the symptoms of atrophic gastritis to return. It is hypothesized that intestinal metaplasia in chronic gastritis caused by *H. pylori* is less likely to improve with *H. pylori* eradication than atrophic gastritis alone. The comparative risk of getting GC following the removal of *H. pylori* was 0.65, according to a meta-analysis. While extensive intestinal metaplasia occurs, there is little data to suggest that treating the *H. pylori* infection lowers the risk of GC[22]. Yan *et al*[23] recently completed a randomized, placebo-controlled study to assess the long-term impact of *H. pylori* eradication medication on the incidence and death of GC in a high-risk group. A total of 1630 asymptomatic *H. pylori*-infected people were randomly allocated to undergo conventional triple treatment for *H. pylori* eradication (n = 817) or a placebo (n = 813), and were then followed up for 26.5 years. There were 35 people in the placebo group (4.31%) and 21 people (2.57%) in the treatment group who tested positive for GC. *H. pylori* medication patients had a lower chance of developing GC in comparison to the placebo group [hazard ratio (HR): 0.57; 95%CI: 0.33-0.98]. They concluded that eradicating *H. pylori* may provide long-term protection against GC in high-risk groups, particularly in infected individuals who did not have precancerous gastric lesions at baseline.

MOLECULAR GENETIC

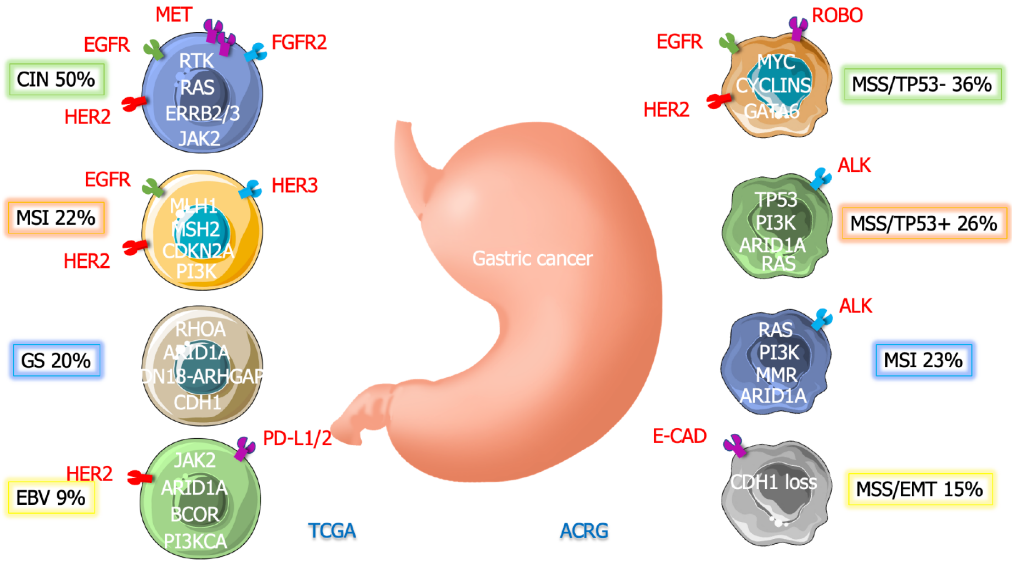
GC is a molecularly diverse malignancy with several genetic mutations. Based on histological results, the Lauren classification originally divided GC into two types (intestinal and diffuse). However, it is unable to reliably predict treatment outcomes and prognosis because it fails to take into consideration the variable nature of GC[24]. The Cancer Genome Atlas (TCGA), by classifying patients into four distinct molecular subtypes based on six different molecular subtypes, has provided a detailed depiction of the genetic underpinnings of GC: (1) Tumors positive for EBV, which display recurrent PIK3CA mutations, extreme DNA hypermethylation, and amplification of Janus-associated kinase 2, programmed cell death ligand-1 (PD-L1), and PD-L2; (2) microsatellite unstable tumors have high mutation rates, including changes in the genes producing proteins that can be targeted to cause cancer; (3) genomically stable tumors that are enriched for mutations in Ras homolog family member A or fusions involving RHO family GTPase-activating proteins as well as diffuse histological variation; and (4) chromosomally unstable tumors with pronounced aneuploidy and localized receptor tyrosine kinase amplification (Table 1)[25]. Moreover, because of the complexity of GC, the Asian Cancer Research Group (ACRG) subtypes were introduced to enhance classification[26-28]. Although the TCGA classification is extensive and provides clinically useful information, no classification method includes all clinically meaningful signals. This would be required to best lead a customized strategy (Figure 1).

Another group, ACRG, developed a different categorization scheme by dividing GC into four subgroups based on gene expression data: Microsatellite stable with tumor protein 53 (TP53) functional loss (MSS/TP53-), MSS/TP53+ (TP53 intact), MSS/Epithelial-mesenchymal transition (EMT) (EMT signatures), and microsatellite instability (MSI) (23%)[7]. The MSI group from TCGA exhibited similarities to the MSI subtype from ACRG. Despite the fact that the EBV-positive, genomically stable, and chromosomal instability subtypes in TCGA were somewhat enriched in the MSS/TP53+, MSS/EMT, and MSS/TP53-subtypes in ACRG, respectively, there were still a number of differences seen in other subtypes. This demonstrates the distinctiveness of these two classes from TCGA and ACRG. The ACRG also included survival data, which showed the prognostic efficacy of each subtype categorization, in contrast to the TCGA. Following MSS/TP53+, MSS/TP53-, and MSS/EMT GC, MSI GC was shown to have the highest overall survival (OS) and the lowest frequency of recurrence[29]. There has been evidence of ethnic influences on molecular features. Despite the fact that the TCGA data did not reveal any significant biological differences between East Asian and other populations, certain variations in pathway-level gene expression were detected. For instance, East Asian patients had

Table 1 The Cancer Genome Atlas has presented a thorough depiction of the molecular basis of gastric cancer[25]

Subtypes	EBV-positive	MSI	GS	CIN
Frequency, %	8.8	21.7	19.7	449.8
Demographic	Male patients (81%)	Old age (median 72 yr)	Young age (median 59 yr)	Not specific
Histology	Not specific	Not specific	Diffuse	Intestinal
Main location	Fundus and body	Fundus, body, and antrum	Mostly diffuse subtype	Majority of tumors at the GEJ
Molecular alterations	EBV-CIMP: (1) PD-L1/2, JAK2 overexpression; (2) Mutation in PIK3CA, ARID1A, and BCOR; (3) CDKN2A silencing; (4) Immune cell signaling; and (5) Rare TP53 mutations	Gastric-CIMP: (1) Hypermutation in TP53, PIK3CA, ERBB2/3, and ARID1A; (2) MLH1 silencing; (3) Mitotic pathways activation; and (4) Commune changes in the genes of *CMH1*	(1) *CDH1* and *RHOA* mutation; (2) CLDN18-ARHGAP fusion; (3) Cell adhesion; (4) Angiogenesis pathways enriched; and (5) Rare *TP53* mutations	(1) TP53 mutation; (2) RTK-RAS activation; and (3) Mutations of SMAD4 and APC
Potential therapeutic points	(1) PIK3CA; (2) JAK2; and (3) PD-L1/L2	(1) PIK3CA; (2) ERBB2/3; (3) EGFR; (4) PD-L1; and (5) MLH1 silencing	(1) RHOA; and (2) CLDN18	(1) RTKs; (2) EGFR; (3) VEGFA; (4) CCNE1; (5) CCND1; and (6) CDK6

APC: Adenomatous polyposis coli; ARHGAP: Rho GTPase activating protein; ARID1A: AT-rich interactive domain-containing protein 1A; BCOR: B-cell lymphoma 6 corepressor; CCND1: Cyclin-D1; CCNE1: Cyclin-E1; CDH1: Cadherin 1; CDK6: Cyclin-dependent kinase 6; CDKN2A: Cyclin-dependent kinase inhibitor 2A; CIMP: CpG island methylator phenotype; CIN: Chromosomal instability; CLDN18: Claudin-18; CMH1: Hypertrophic cardiomyopathy-1; GS: Genomically stable; GC: Gastric cancer; EBV: Epstein-Barr virus; EGFR: Epithelial growth factor receptor; ERBB2/3: Erb-b2 receptor tyrosine kinase 2/3; GEJ: Gastroesophageal junction; JAK2: Janus-associated kinase 2; MLH1: MutL protein homolog 1; MSI: Microsatellite instability; PD-L1/2: Programmed death ligand-1/2; PI3K: Phosphatidylinositol-3-kinase; RAS: Rat sarcoma virus; RHOA: Ras homolog family member A; RTKs: Receptor tyrosine kinases; SMAD4: Mothers against decapentaplegic homolog 4; TP53: Tumor protein 53; VEGFA: Vascular endothelial growth factor A.



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Figure 1 The molecular findings in gastric cancer by The Cancer Genome Atlas compared with the Asian Cancer Research Group. TCGA: The Cancer Genome Atlas; ACRG: Asian Cancer Research Group; EGFR: Epithelial growth factor receptor; CIN: Chromosomal instability; HER: Human epidermal growth factor receptor; FGFR2: Fibroblast growth factor receptor 2; MSI: Microsatellite instability; GS: Genomically stable; EBV: Epstein-Barr virus; PD-L1/2: Programmed death ligand-1/2; RTKs: Receptor tyrosine kinases; RAS: Rat sarcoma virus; ERBB2/3: Erb-b2 receptor tyrosine kinase 2/3; JAK2: Janus-associated kinase 2; MLH1: MutL protein homolog 1; MSH: Melanocyte-stimulating hormones; CDKN2A: Cyclin-dependent kinase inhibitor 2A; PI3K: Phosphatidylinositol-3-kinase; ARID1A: AT-rich interactive domain-containing protein 1A; BCOR: B-cell lymphoma 6 corepressor; MSS: Microsatellite stable; TP53: tumor protein 53; MYC: Myelocytomatosis oncogene; GATA6: GATA binding protein 6; MMR: Measles-mumps-rubella; CDH1: Cadherin 1; EMT: Epithelial-mesenchymal transition; E-CAD: E-cadherin; ALK: Anaplastic lymphoma kinase.

elevated telomerase regulatory pathway expression and decreased hypoxia inducible factor-1-alpha transcription factor network expression[29]. Another study found that Asian and non-Asian GC patients had significantly different tumor immunity profiles. Non-Asian cases of GC were connected to an enrichment of T-cell gene expression patterns and a lesser expression of the immunosuppressive marker FOXP3 as compared to Asian cases of GC[30-32]. Further research with a sufficient sample size is

required to better understand how racial variations affect molecular background.

ANTITUMOR IMMUNE RESPONSES

Host immunity is now widely recognized as being important in the prevention of cancer. The findings suggest that our immune system can inhibit cancer growth through a mechanism known as immune surveillance. Dying cancer cells may generate and disseminate tumor-specific and tumor-related antigens that can be ingested and processed by tissue-resident dendritic cells (DCs). These cells then develop into antigen-presenting cells (APCs) in the presence of a favorable microenvironment, which is typically rich in activator molecules known as danger-associated molecular patterns, which are released by dying cancer cells[33,34]. Mature APCs must effectively deliver tumor antigens in the form of peptides to CD8+ T lymphocytes *via* major histocompatibility complex (MHC) class I molecules and CD4+ T lymphocytes *via* MHC class II molecules in order to trigger effective anticancer immunity. The most effective tumor antigens are non-self or altered proteins, such as those produced by somatic mutations in genes expressed by tumor cells or those encoded by viruses. Effective activation of CD8+ T cells requires both antigen presentation as a first signal and the presence of costimulatory molecules as a second signal. Once these cells are activated, they enter the tumor bed and multiply. Furthermore, T lymphocytes are able to recruit other immune cells, such as natural killer (NK) cells and M1 macrophages, that are able to further aid in the destruction of cancer cells. Additionally, T lymphocytes themselves are able to directly destroy cancer cells through the production of cytokines. This process is essential for the body to effectively fight cancer cells. The fact that not all traditional cytotoxic chemotherapeutic medicines are immunosuppressive is important in light of the practical use of immunotherapeutic methods. Recent research has revealed that a number of medications commonly used in medical practice may have the ability to kill tumor cells *via* an immunogenic cell death pathway. This pathway has the potential to activate powerful innate and adaptive anticancer immune responses. Medications found to have this effect include doxorubicin, mitoxantrone, bortezomib, oxaliplatin, and cyclophosphamide. Such findings have the potential to revolutionize cancer treatments, offering new hope for cancer patients across the world[35,36].

CANCER CELL IMMUNOESCAPE

T-cell receptor (TCR), co-stimulatory molecules, including CD28, and cytokines are all required for effective activation of cytotoxic T cells. Tumor cells can resist immunosuppression through a variety of mechanisms. The production of several co-inhibitory receptors is one of the ways to prevent T cell activation in the body. PD-1, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), and lymphocyte-activation gene 3 (LAG-3) are examples of inhibitory receptors that bind to tumor cell ligands and block T cell activation. The overexpression of these receptors induces the inhibition of coinhibitory pathways, such as the B7-CD28-CTLA-4 family. This helps to ensure the physiological functioning of the immune system, which is essential in preventing tumor growth and the spread of cancer cells[37,38]. CTLA-4 is an important co-stimulatory molecule expressed on the surface of CD4+ and CD8+ T lymphocytes. It binds to molecules known as CD80 (B7-1) and CD86 (B7-2) on APCs, which are involved in the initiation and regulation of adaptive immunity. The binding of CTLA-4 to CD80 and CD86 on APCs results in the inhibition of T cell activation. Therefore, this molecule plays an important role in regulating the immune system. This inhibits TCR signaling by preventing APCs from binding to the CD28 co-stimulatory molecule. Additionally, CTLA-4 is continually produced in regulatory T cells (Treg) in order to control their immunosuppressive role. Tumor cells are thought to escape through downregulation of MHC class I expression. MHC class I molecules play a significant role in cytotoxic T cell-mediated immunity. Tumor cells have the ability to suppress immune cell activity by secreting immunosuppressive substances. These substances, such as interleukin-10, transforming growth factor-β1, galectins, tumor necrosis factor, prostaglandin E2, and vascular endothelial growth factor (VEGF), can inhibit the function of the immune cells, thus disrupting the body's natural defense system. The immunosuppressive substances also inhibit the receptors on the surface of the immune cells, making them unable to recognize and attack the tumor cells. This allows the tumor cells to grow and spread unchecked. Therefore, it is important to understand the role of these immunosuppressive substances in order to develop effective treatments for cancer[39].

By encouraging the polarization towards less cytotoxic T cell subsets and pro-inflammatory T cell subsets, the tumor microenvironment may compromise anti-tumor immunity. TH-2, TH-17, and Treg are T helper (TH) cells that are related to tumors. Tumor-associated macrophages (TAM) make up the majority of the immune cell population in the tumor microenvironment. Two separate subtypes of these macrophages, M1 and M2, exhibit anti-tumor and pro-tumorigenic actions, respectively. When a GC site was infiltrated with M2 TAM, the patient's prognosis was usually poor[40,41]. Myeloid-derived suppressor cells (MDSCs) are a diverse population of cells with the capacity to proliferate vigorously under pathological conditions like cancer. They are descended from the myeloid lineage. Both innate

and adaptive immunity against malignancies can be suppressed by these cells. Recent studies have confirmed that patients with GC are more likely to have an increased number of MDSCs. This increased presence of MDSCs in the blood samples of GC patients has been associated with poorer clinical result [42,43]. Treg cells are a significant contributor to immune suppression in the tumor microenvironment. End-stage instances of GCs were replete with Foxp3 plus CD4 plus ICOS plus effector Tregs, also known as highly suppressive Tregs[44,45].

AGC IMMUNOTHERAPY

Patients with GC exhibit "three high and three low" characteristics: High rates of incidence, metastatic condition, and mortality; low rates of early discovery, radical resection, and five-year survival. Patients might either be early-stage or advanced-stage patients. Early-stage GCs are limited to the mucosa or submucosa, regardless of the size of the lesion or the presence of lymph node metastases. AGC is defined as cancers that infiltrate into or beyond the subserosa to surrounding organs or metastasize. Middle GC is described as cancer that extends past the submucosa to penetrate the gastric muscle layer. Tumors in the advanced stage of GC include intermediate and advanced tumors. Another classification of AGC includes local unresectable GC, distant metastasis, and postoperative recurrent GC[46,47]. The therapy's efficacy and method are determined by the tumor's stage. At the moment, the primary objective of treating AGC is to ameliorate symptoms and extend patients' survival times using successive courses of chemotherapy. Systemic chemotherapy alone is becoming increasingly effective in treating AGC, yet the median survival duration with this approach is still only 4-13 mo. This demonstrates that, unfortunately, the prognosis for this disease is far from favorable. In spite of the progress made in treating AGC with chemotherapy, more research and development are needed to improve the outlook for those afflicted with it. As a result, both more effective chemotherapy medications and regimens with fewer hazardous side effects, as well as innovative therapeutic paradigms, such as targeted therapy and immunotherapy, should be investigated.

Immunotherapy is a form of cancer treatment that employs the body's own immune system to fight cancer cells. It is divided into two types: Passive and active. Passive immunotherapy is a form of therapy that utilizes antibody therapies to target cancer cells. On the other hand, active immunotherapy focuses on boosting the body's immune response against tumor cells. Examples of active immunotherapy include vaccinations and chimeric antigen receptors (CAR). CARs are designed to recognize and bind to tumor cells, triggering the body's immune system to attack the cancer cells. However, with passive immunotherapy, immune system components, such as mAbs, are generated outside of the body. Immunotherapies nowadays are frequently based on cytotoxic T cells, mAbs, and gene-transfected vaccines. Immune checkpoint inhibitors (ICIs) have become more popular since ipilimumab was originally approved in 2011 for the treatment of metastatic BRAF-negative melanoma. Following more than 1000 clinical investigations, ICIs are now recognized as a therapeutic approach in the management of both solid organ and hematologic malignancies[48-50].

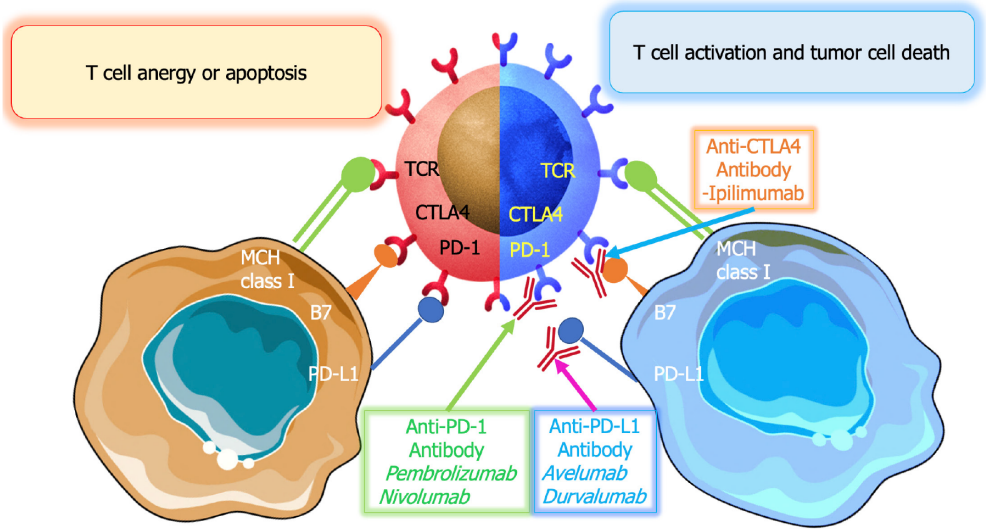
PASSIVE IMMUNOTHERAPY

ICIs

ICIs are a type of drug used in cancer immunotherapy. They work by preventing the T cells from the immune system from being suppressed by the tumor. Targeting CTLA-4, PD-1, and PD-L1, three essential ICI drug types were previously created for preclinical and clinical research. In order to lessen or decrease CD28 signaling, CTLA-4, a CD28 homolog, may bind to B7-1 and B7-2 with a greater affinity than CD28. However, PD-1 encourages tumor cell survival by inducing apoptosis in T lymphocytes that have been activated. Pharmacological blockage of its route may damage the function of immune cells such as B cells and NK cells since PD-1 is extensively expressed in many immune cells[51-53] (Figure 2).

Pembrolizumab

Pembrolizumab was the first monoclonal antibody designed to target PD-1. In 2017, the FDA authorized it for the treatment of advanced non-small cell lung cancer. In a phase I study, the anti-tumor efficacy, safety, and tolerability of pembrolizumab were assessed in 39 patients with AGC (KEYNOTE-012). According to the findings, pembrolizumab provides promising anti-tumor effectiveness with a manageable amount of toxicity in these individuals. The positive outcomes of this experiment spurred the conduct of more clinical trials testing PD-1-blocking therapy[52]. In 2018, Fuchs *et al*[53] performed the KEYNOTE-059 trial, a phase 2 global, open-label, single-arm, multicohort study that recruited 259 AGC patients from 16 countries to assess the safety and effectiveness of pembrolizumab. They discovered an 11.6% objective response rate (ORR) with a 2.3% full response rate. The median response time was 8.4 mo. ORR and median response duration were 15.5% and 16.3 mo in PD-L1-positive patients, respectively, compared to 6.4% and 6.9 mo in PD-L1-negative patients. One or more grade 3-5



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Figure 2 Immune check point inhibitors for advanced gastric cancer treatment. CTLA4: Cytotoxic T-lymphocyte-associated protein 4; TCR: T-cell receptor; PD-1: Programmed cell death protein 1; PD-L1: Programmed cell death ligand-1; MCH: Melanin-concentrating hormone.

treatment-related adverse events (TRAEs) occurred in 46 individuals (17.8%). Two patients (0.8%) stopped due to TRAEs, and two fatalities were deemed treatment-related. They concluded that in patients with AGC or advanced gastroesophageal junction cancer (AGEJC) who had previously undergone at least two lines of treatment, Pembrolizumab monotherapy displayed promising efficacy and manageable safety. Patients with PD-L1-positive malignancies experienced long-term responses. Furthermore, Shitara *et al*[54] undertook a randomized, open-label, phase 3 research study in 30 countries to evaluate Pembrolizumab *vs* Paclitaxel in 592 patients with AGC or AGEJC who had progressed on platinum- and fluoropyrimidine-based first-line treatment. With a combined positive score (CPS) of 1 or higher, they found that 326 people passed away (151 of 196 patients in the Pembrolizumab group and 175 of 199 patients in the Paclitaxel group). With Pembrolizumab, the median OS was 9.1 mo, while with Paclitaxel, it was 8.3 mo. With Pembrolizumab, the median progression-free survival (PFS) was 1.5 mo, while with Paclitaxel, it was 4.1 mo. Furthermore, grade 3-5 TRAEs occurred in 42 (14%) of the 294 patients treated with Pembrolizumab and 96 (35%) of the 276 patients treated. They concluded that Pembrolizumab, when used as a second-line treatment for AGC or AGEJC with a PD-L1 CPS of 1 or higher, did not substantially increase OS when compared to Paclitaxel. However, Paclitaxel has a worse safety profile than Pembrolizumab (Table 2).

Bang *et al*[55] conducted the KEYNOTE-059 multicohort, phase 2, non-randomized trial in 56 patients with AGC or AGEJC in 2019. They discovered that the ORR was 60.0% in patients receiving combination treatment and 25.8% in individuals receiving Pembrolizumab monotherapy. Also, in the combination therapy cohort, 19 patients (76.0%) experienced grade 3/4 treatment-related side events; none were fatal. In the monotherapy cohort, seven patients (22.6%) experienced grade 3-5 TRAEs; one fatality was ascribed to a TRAE (pneumonitis). They concluded that in patients with previously untreated AGC or AGEJC, Pembrolizumab displayed anticancer efficacy and was well tolerated as monotherapy and in combination with chemotherapy. One year later, Kawazoe *et al*[56] performed a non-randomized, multicenter, open-label phase IIb study, KEYNOTE-659, in 54 AGC or AGEJC patients with human epidermal growth factor receptor 2-negative and PD-L1-positive to assess the safety and efficacy of Pembrolizumab combined with chemotherapy [S-1 plus oxaliplatin (SOX)] as the first-line treatment. They observed that the ORR and disease control rate (DCR) were, respectively, 72.2% and 96.3%. In terms of DOR, time to response (TTR), PFS, and OS, the median values were not attained at 1.5 mo, 9.4 mo, or not reached at all. Patients with CPSs of 1 to 10 had an ORR of 73.9%, whereas those with CPSs of more than 10 had an ORR of 70.0%. Grade 3 TRAEs occurred in 57% of participants. The most frequent grade 3 adverse events were decreased platelet count (14.8%), decreased neutrophil count (13.0%), colitis (5.6%), and adrenal insufficiency (5.6%). They came to the conclusion that SOX and Pembrolizumab demonstrated potential efficacy and a manageable safety profile for the first management of AGC or AGEJC.

Pembrolizumab was also examined in conjunction with a targeted therapy such as Lenvatinib in an open-label, single-arm, phase 2 study in 29 patients with AGC to assess the combination of Lenvatinib and Pembrolizumab. They discovered that 69% of the patients had an ORR. Hypertension, proteinuria, and a fall in platelet count were the most frequently occurring grade 3 TRAEs, occurring in 11 (38%) of the patients, five (17%) of the patients, and two (7%) of the patients, respectively. No grade 4 adverse events, no serious adverse events, and no fatalities associated with the therapy were reported. They

Table 2 Immunotherapies for advanced gastric cancer patients

Ref.	Drug(s)	Number of patients	Study phase	ORR (%)	Median OS (months)	Median PFS (months)	Results
Muro *et al* [52]	Pembrolizumab	39	1b	33	11.4	1.8	Pembrolizumab demonstrated a reasonable safety profile and potential antitumor efficacy in metastatic PD-L1-positive GC, warranting further exploration in phase 2 and 3 studies
Fuchs *et al* [53]	Pembrolizumab	259	2	15.5	5.6	2.0	Pembrolizumab is a potential new therapy option for AGC or AGEJC that has progressed following second-line treatment, demonstrating high and persistent responses. Pembrolizumab has a mechanism of action, duration of response, and toxicity profile that differs from and does not overlap with conventional treatment for gastroesophageal adenocarcinoma
Shitara *et al* [54]	Pembrolizumab *vs* Paclitaxel	395	3	-	9.1/8.3 (Pem/Pac)	1.5/4.1 (Pem/Pac)	When compared to Paclitaxel, Pembrolizumab did not significantly improve overall survival when administered as a second-line therapy for AGC or AGEJC with PD-L1 CPS of 1 or higher
Bang *et al* [55]	Pembrolizumab *vs* Pembrolizumab plus chemotherapy	56	2	25.8/60.0 (Pem/Pem+Chem)	13.8/20.7 (Pem/Pem+Chem)	3.3/6.6 (Pem/Pem+Chem)	Pembrolizumab combined chemotherapy showed acceptable tolerability and potential anticancer efficacy in AGC or AGEJC, independent of PD-L1 expression. In patients with PD-L1 CPS ≥ 1, pembrolizumab monotherapy revealed good antitumor efficacy and acceptable safety
Kawazoe *et al*[56]	Pembrolizumab plus chemotherapy	54	2b	72.2	Not reached	9.4	For the first-line treatment of AGC or AGEJC patients, chemotherapy with Pembrolizumab shown good effectiveness and a tolerable toxicity profile
Kawazoe *et al*[57]	Pembrolizumab plus Lenvatinib	29	2	69.0	Not reached	7.1	In patients with AGC, the combination of Lenvatinib and Pembrolizumab demonstrated promising anti-tumor effectiveness while maintaining a tolerable safety profile
Shitara *et al* [58]	Pembrolizumab *vs* Pembrolizumab plus chemotherapy *vs* chemotherapy	763	3	-	10.6/11.1 (Pem/chem) CPS ≥ 1; 17.0/10.8 CPS ≥ 10		In individuals with untreated AGC or AGEJC, Pembrolizumab was shown to be noninferior to chemotherapy, with less adverse effects. Pembrolizumab alone or in combination with chemotherapy did not outperform treatment in terms of OS and PFS
Kwon *et al* [60]	Pembrolizumab	18	2	55.6	-	-	A subset of MSI-H GC patients with certain immunological responses at baseline, such as stronger TMB, abundant T cell infiltration, more TCR clonal diversity, and less stem-like exhausted T cells, may not require anything more than anti-PD-1 monotherapy

Yamaguchi *et al*[63]	Pembrolizumab plus SOX *vs* Pembrolizumab plus SP	100	2b	72.2/80.4	16.9/17.1	9.4/8.3	In Japanese patients with PD-L1 positive, HER-2 negative AGC or AGEJC, the combination of Pembrolizumab plus SOX or SP as first-line treatment indicated high efficacy and reasonable tolerability
Lee *et al*[64]	Pembrolizumab plus Trastuzumab plus Capecitabine plus Cisplatin	43	1b/2	76.7	19.3	8.6	The use of a quadruplet combination as first-line therapy (Pembrolizumab plus Trastuzumab plus Capecitabine plus Cisplatin) resulted in tumor decrease in HER-2-positive AGC
Satake *et al* [65]	Pembrolizumab *vs* Pembrolizumab plus chemotherapy *vs* chemotherapy	187	3	22.6/37.7 (Pem/chem); 26.9/31.8 (PD-L1 CPS ≥ 10)	22.7/13.8 (Pem/chem); 28.5/14.8 (PD-L1 CPS ≥ 10)	4.1/6.5 (Pem/chem); 7.2/6.9 (PD-L1 CPS ≥ 10)	Pembrolizumab monotherapy was related with statistically better OS results in patients with AGC or AGEJC with PD-L1 CPS ≥ 1 and CPS ≥ 10 tumors as compared to chemotherapy alone. When compared to chemotherapy, Pembrolizumab monotherapy had a better tolerability profile
Janjigian *et al*[66]	Nivolumab plus Ipilimumab *vs* Nivolumab	160	1/2	24/12 (Niv+Ipi/Niv)	6.9/6.2 (Niv+Ipi/Niv)	1.6/1.4 (Niv+Ipi/Niv)	Nivolumab and Nivolumab in combination with Ipilimumab provide a viable treatment option for individuals with AGEJC
Kang *et al* [67]	Nivolumab *vs* placebo	493	3	11.2/0.0	5.32/4.14	1.61/1.45	Nivolumab might be a potential therapy option for people with AGC or AGEJC who have been highly pretreated
Boku *et al* [69]	Nivolumab plus SOX *vs* Nivolumab plus CapeOX	77	2	57.1/76.5	Not reached	9.7/10.6	In these individuals, Nivolumab in conjunction with SOX or CapeOX was well tolerated and showed potential efficacy
Nakajima *et al*[71]	Nivolumab plus Paclitaxel plus Ramucirumab	43	1/2	37.2	13.1	5.1	As a second-line treatment for AGC, Nivolumab in combination with Paclitaxel and Ramucirumab shown promising antitumor activity with tolerable tolerability
Janjigian *et al*[72]	Nivolumab plus chemotherapy *vs* chemotherapy	1,581	3	51/41	14.4/11.1	7.7/6.1	Nivolumab in conjunction with chemotherapy is being considered as a new standard first-line treatment for these individuals
Shah *et al* [73]	Andecaliximab plus Nivolumab *vs* Nivolumab	141	2	10/7	7.1/5.9	-	When compared to Nivolumab alone, the combination of Andecaliximab and Nivolumab exhibited a favorable safety profile but did not boost efficacy in these people
Kang *et al* [74]	Nivolumab plus oxaliplatin-based chemotherapy *vs* placebo plus oxaliplatin-based chemotherapy	724	2/3	-	17.45/17.15	10.45/8.34	In these patients, Nivolumab in conjunction with oxaliplatin-based chemotherapy improved PFS but not OS
Bang *et al* [76]	Avelumab *vs* chemotherapy	371	3	2.2/4.3	4.6/5.0	1.4/2.7	As compared to chemotherapy, treating these patients in the third-line setting with single-agent Avelumab did not improve OS or PFS. Avelumab, on the other hand, had a more manageable toxicity profile than chemotherapy
Moehler *et*	Avelumab *vs*	499	3	-	10.4/10.9	-	In patients with AGC or AGEJC

al[77]	chemotherapy	in general, or in a specified PD-L1-positive population, Avelumab maintenance therapy did not give a superior OS when compared to continuing chemotherapy

AGC: Advanced gastric cancer; AGEJC: Advanced gastroesophageal junction cancer; CapeOX: Capecitabine plus oxaliplatin C; Chem: Chemotherapy; CPS: Combined positive score; HER-2: Human epidermal growth factor receptor 2; MIS-H: High microsatellite instability; Niv: Nivolumab; Niv+Ipi: Nivolumab plus Ipilimumab; ORR: Objective response rate; OS: Overall survival; Pac: Paclitaxel; Pem: Pembrolizumab; PD-L1: Program cell death ligand 1; PFS: Progression-free survival; SOX: S-1 plus oxaliplatin; SP: S-1 and cisplatin; TCR: T-cell receptor; TMB: Tumor mutational burden.

concluded that Lenvatinib and Pembrolizumab had potential anti-tumor effectiveness with a tolerable safety profile in AGC patients[57]. A phase 3 KEYNOTE-062 randomized, controlled, and partially blinded interventional trial was carried out by Shitara *et al*[58] in 2020 in 763 patients from 29 countries who had untreated AGC or AGEJC and a PD-L1 CPS of 1 or above. Every three weeks, participants were given a random choice between receiving Pembrolizumab 200 mg, chemotherapy plus placebo, or chemotherapy combined with cisplatin 80 mg/m^2/d on day 1 plus fluorouracil 800 mg/m^2/d on days 1 through 5. After a median follow-up of 29.4 mo (median OS, 10.6 *vs* 11.1 mo), they found that Pembrolizumab was non-inferior to chemotherapy for OS in patients with a CPS of 1 or higher. Chemotherapy was not better than Pembrolizumab monotherapy in individuals with a CPS of 1 or higher. Patients with a CPS of 10 or higher experienced longer OS with pembrolizumab compared to chemotherapy (median OS, 17.4 *vs* 10.8 mo); however, the difference was not statistically significant. In terms of OS in patients with a CPS of 1 or higher (12.5 *vs* 11.1 mo), CPS of 10 or greater (12.3 *vs* 10.8 mo), or PFS in patients with a CPS of 1 or higher (6.9 *vs* 6.4 mo), Pembrolizumab in combination with chemotherapy did not outperform treatment. Pembrolizumab was found to be noninferior to chemotherapy in patients with untreated AGC or AGEJC, with fewer side effects. For the OS and PFS end points assessed, Pembrolizumab or Pembrolizumab with chemotherapy were not superior to chemotherapy. Additionally, Pembrolizumab was evaluated in PD-L1-positive (CPS ≥ 10) AGC or AGEJC patients from KEYNOTE-062 (n = 182), KEYNOTE-061 (n = 108), and KEYNOTE-059 (n = 46) to better define the specificity of CPS as a predictor of clinical outcomes. This thorough study found that pembrolizumab improved clinical outcomes in patients with CPS ≥ 10 AGC or AGEJC across many lines of treatment[59].

In 2021, Kwon *et al*[60] conducted a phase 2 study of Pembrolizumab in 18 patients with advanced high MSI (MSI-H) GC, including serial and multi-region tissue samples as well as serial peripheral blood testing, with a median follow-up of 19.5 mo. The findings showed that 6 patients (33.3%) had stable disease, 3 patients (16.7%) had a complete response (CR), 7 patients (38.9%) had a verified partial response (PR), and 3 patients (16.7%) had a CR, giving an ORR of 55.6% and a DCR of 88.9%. They proposed that a subgroup of MSI-H GC patients with a specific immunological response, as defined by a higher tumor mutational burden (TMB), abundant T cell infiltration, larger TCR clonal diversity, and fewer stem-like exhausted T cells at baseline, may not require anything more than anti-PD-1 monotherapy. Equally significant clinically was the finding of unfavorable genomic and immunologic characteristics from the outset, revealing a subset of MSI-H GC that may require additional therapy to benefit from PD-1 blocking. These findings indicated a combination therapy aimed at lowering Treg populations and/or augmenting and growing NK-cell numbers in this fraction. Synthetic model systems that mimic MSI-H biology, as well as extensive genomic and immunologic screening for therapeutic vulnerabilities, will be critical in identifying potential combinations for future testing. To stratify MSI-H cancers for therapy with either PD-1 blockade alone or cutting-edge combination approaches, the findings, however, signal that accurate pre- and post-treatment characterizations are attainable and will probably be needed.

When used as second-line therapy in the phase 3 KEYNOTE-061 study for patients with PD-L1 CPS > 1 AGC or AGEJC, Pembrolizumab did not significantly increase OS compared with Paclitaxel. Fuchs *et al*[61] conducted a trial in which they randomly assigned patients to receive Pembrolizumab 200 mg Q3W for 35 cycles or standard-dose paclitaxel and presented outcomes in the CPS 1, 5, and 10 populations after two years of follow-up. The findings revealed that 366 of 395 individuals (92.7%) with CPS ≥ 1 died. In the CPS ≥ 1 cohort, Pembrolizumab showed a tendency toward increased OS *vs* Paclitaxel; 24-mo OS rates: 19.9% *vs* 8.5%. With PD-L1 enrichment, Pembrolizumab gradually increased the OS benefit (CPS > 5: 24-mo rate, 24.2% *vs* 8.8%; CPS > 10: 24-mo rate, 32.1% *vs* 10.9%). Across treatment groups, the median PFS was similar (CPS > 1: HR, 1.25; CPS > 5: 0.98; CPS > 10: 0.79). The median DOR was 19.1 *vs* 5.2 mo, 32.7 *vs* 4.8 mo, and NR *vs* 6.9 mo; the ORR (Pembrolizumab *vs* Paclitaxel) was 16.3% *vs* 13.6% (CPS > 1), 20.0% *vs* 14.3% (CPS > 5) and 24.5% *vs* 9.1% (CPS > 10). Pembrolizumab was associated with fewer TRAEs than Paclitaxel (53% *vs* 84%). In 94 Asian patients with advanced PD-L1-positive (CPS > 1) AGC or AGEJC, 36 medical centers in China, Malaysia, South Korea, and Taiwan conducted the randomized, open-label, phase 3 study KEYNOTE-063, which compared Pembrolizumab *vs* Paclitaxel as second-line therapy. The results revealed that the median OS in Pembrolizumab plus Paclitaxel therapy was the same as 8 mo. The median PFS with Pembrolizumab

was 2 mo *vs* 4 mo with Paclitaxel. Pembrolizumab had a 13% ORR against Paclitaxel's 19%. Any-grade TRAEs occurred in 28 patients receiving Pembrolizumab (60%) and 42 patients receiving Paclitaxel (96%), respectively; grades 3-5 events occurred in 5 patients (11%) and 28 patients (64%). They stated that, due to inadequate power, decisive conclusions concerning the effectiveness of second-line Pembrolizumab in Asian patients with advanced PD-L1-positive AGC or AGEJC are restricted, however, Pembrolizumab was well tolerated in this patient population. Efficacy followed a similar pattern to that shown in the phase 3 KEYNOTE-061 experiment[62].

Yamaguchi *et al*[63] conducted an open-label phase 2b study, KEYNOTE-659, in Japan in 2022 to examine the effectiveness and safety of first-line Pembrolizumab plus SOX (cohort 1, n = 54) or S-1 and cisplatin (SP) (cohort 2, n = 46) for AGC or AGEJC. They reported that the median duration of Pembrolizumab therapy in cohorts 1 and 2 was 6.0 and 5.1 mo, respectively. SOX (cohort 1) had a median treatment length of 4.9 mo, while SP (cohort 2) had a median treatment duration of 4.4 mo. In cohort 1, 35 patients (64.8%) had their S1 dosage reduced, 47 patients (87.0%) had their oxaliplatin dose reduced, 44 patients (81.5%) had their S1 dose interrupted, and 31 patients (57.4%) had their oxaliplatin dose interrupted. In cohort 2, 33 patients (71.7%) had their S1 dosage reduced, 43 patients (93.5%) had their cisplatin dose reduced, 29 patients (63.0%) had their S1 dose interrupted, and 22 patients (47.8%) had their cisplatin dose interrupted. The ORR in cohort 1 was 72.2% (39 of 54 patients) and 80.4% (37 of 46 patients) in cohort 2. Overall, tumor reduction was observed in 52 of 54 patients (96.3%) in cohort 1 and 44 of 46 patients (95.7%) in cohort 2. DCR in cohort 1 was 96.3% (52 of 54 patients) and 97.8% (45 of 46 patients) in cohort 2. The median PFS in cohorts 1 and 2 was 9.4 mo and 8.3 mo, respectively. In cohort 1, the median OS was 16.9 mo, while in cohort 2, it was 17.1 mo. The median DOR in cohort 1 was 10.6 mo and 9.5 mo in cohort 2, whereas the median TTR in both cohorts was 1.5 mo. They proposed that the combination of Pembrolizumab with SOX or SP as first-line therapy in Japanese patients with PD-L1 positive, Human epidermal growth factor receptor (HER)-2 negative, AGC, or AGEJC demonstrated good effectiveness and tolerable safety.

Furthermore, 43 HER-2-positive AGC patients with a median follow-up of 18.2 months underwent Pembrolizumab evaluation in a single-arm, multi-institutional phase 1b/2 research to evaluate a quadruplet combination of Pembrolizumab, Trastuzumab, Capecitabine, and Cisplatin as first-line therapy. They reported an ORR of 76.7%, with 27 (62.8%) exhibiting PR and six (14.0%) patients exhibiting CR. Nine patients (20.9%) had stable disease, and the DCR was 97.7%. In 37 patients (86.0%), the total tumor burden was reduced by 30%, and in 26 (56.6%), it was reduced by 50%. The median PFS was 8.6 mo, with a 79.1% 6-mo PFS rate and a 41.9% 1-year PFS rate. The median OS was 19.3 mo, with an 80.1% 1-year OS rate. The median number of treatment cycles was 12. They concluded that utilizing a quadruplet regimen as first-line treatment resulted in tumor reduction in HER-2-positive AGC[64].

Recently, Satake *et al*[65] conducted a randomized control, phase 3 KEYNOTE-062 trial in 187 patients with AGC or AGEJC to compare the effectiveness of Pembrolizumab or Pembrolizumab with chemotherapy *vs* standard of care chemotherapy. They found that in the PD-L1 CPS ≥ 1 patients, the median OS with Pembrolizumab was 22.7 mo compared to 13.8 mo with chemotherapy. The 12-mo and 24-mo OS rates with Pembrolizumab were 69.4% and 44.8%, respectively, compared to 54.1% and 23.0% with chemotherapy. In the PD-L1 CPS ≥ 10 patients, the median OS with Pembrolizumab was 28.5 mo *vs* 14.8 mo with chemotherapy. The 12-mo and 24-mo OS rates with Pembrolizumab were 80.8% and 53.6%, respectively, compared to 68.2% and 27.3% with chemotherapy. They proposed that Pembrolizumab monotherapy was linked with numerically better OS results in patients with AGC or AGEJC with PD-L1 CPS ≥ 1 and CPS ≥ 10 tumors as compared to chemotherapy alone. When compared to chemotherapy, Pembrolizumab monotherapy had a better tolerability profile.

Nivolumab

A humanized immunoglobulin G (IgG) 4 monoclonal anti-PD-1 antibody called Nivolumab is effective against a range of tumor types. The phase 1/2 CheckMate-032 trial compared the use of Nivolumab and Ipilimumab in combination with Nivolumab monotherapy in 160 patients with AGC or AGEJC. The ORR for patients who got Nivolumab and Ipilimumab together was 24% as opposed to 12% for Nivolumab alone. Only 8% of patients in the combination arms responded to the alternate dosage (Nivolumab 3 mg/kg and Ipilimumab 1 mg/kg), suggesting that the ORR in these arms was dose-dependent. Regardless of PD-L1 expression, responses were seen. Nivolumab plus Ipilimumab treatment was linked with more severe toxicity (43%) than nivolumab alone (10%), as predicted from past combination trials[66]. Among 493 patients with unresectable AGC or AGEJC who had shown resistance to or intolerance to two or more prior chemotherapy regimens, ONO-4538-12 (ATTRACTION-2) was a multicenter, double-blind, randomized phase 3 study of Nivolumab. The ICI in GCs was the subject of the initial phase 3 placebo-controlled, randomized study. For the first time, the study demonstrated that PD-1 inhibition can improve OS in patients with severely pre-treated GC. The observed median OS with Nivolumab was 5.32 mo *vs* 4.14 mo with placebo, and the 12-month OS rate was 26.6% *vs* 10.9%[67]. The median PFS with Nivolumab was 1.61 mo *vs* 1.45 mo with placebo. The median time for a response to Nivolumab was 9.53 mo, and the ORR rate with Nivolumab was 11.2% as opposed to 0% with placebo. Nivolumab recipients had a tolerable safety profile, with 34 (10%) out of 330 patients experiencing TRAEs (grade 3 or 4), which is a rate similar to placebo recipients. However, it should be mentioned that the ATTRACTION-2 only included individuals from Asian countries,

therefore, the results might not apply to populations in Europe and North America. Emerging evidence suggests that unique gene profiles related to inflammation and immunity exist in Asian and non-Asian individuals with GC[68].

In order to assess the safety and effectiveness of Nivolumab in combination with SOX or capecitabine plus oxaliplatin (CapeOX) as first-line therapy in 77 patients with unresectable advanced or recurrent HER-2-negative AGC or AGEJC, Boku *et al*[69] conducted a randomized, phase 2 trial known as ATTRACTION-4. They discovered that Nivolumab with SOX resulted in an ORR of 57.1%, and Nivolumab plus CapeOX resulted in an ORR of 76.5%. In both groups, the median OS was not attained. The median PFS was 9.7 mo *vs* 10.6 mo. Neutropenia (14.3%) was the most common grade 3/4 TRAE in the nivolumab plus SOX group, followed by anemia (16.7%), peripheral sensory neuropathy, reduced appetite, type 1 diabetes mellitus, and nausea (11.1%) in the nivolumab with CapeOX group. They concluded that Nivolumab in combination with SOX/CapeOX was well tolerated and showed promising effectiveness in patients with unresectable advanced or recurrent HER-2-negative GC or GEJC. The ATTRACTION-2 2-year follow-up data revealed that 493 of 601 screened individuals were randomized (2:1) to receive Nivolumab (n = 330) or placebo (n = 163), and that the OS was considerably longer in the Nivolumab group compared to the placebo group (5.26 mo *vs* 4.14 mo) at the 2-year follow-up. At 1 year (27.3% *vs* 11.6%) and 2 years (10.6% *vs* 3.2%), the Nivolumab group had a greater OS rate than the placebo group. Regardless of tumor PD-L1 expression, the OS advantage was seen. The median OS for patients in the Nivolumab group who had a full or PR was 26.6 mo; the OS rates at 1 and 2 years were 87.1% and 61.3%, respectively. There were no new safety signals discovered[70]. In a phase 1/2 study, Japanese researchers investigated the safety and effectiveness of Nivolumab with Paclitaxel plus Ramucirumab in 43 patients with AGC resistant to first-line treatment. They discovered an ORR of 37.2% and a 6-mo PFS rate of 46.5%. The median OS was 13.1 mo: 13.8 mo in CPS ≥ 1 patients and 8.0 mo in CPS < 1 patients. They proposed that Nivolumab in combination with Paclitaxel and Ramucirumab showed potential anti-tumor efficacy with acceptable toxicity as a second-line therapy for AGC[71].

Janjigian *et al*[72] conducted a multicenter, randomized, open-label, phase 3 study (CheckMate 649) in 1581 patients with AGC, AGEJC, or esophageal adenocarcinoma (Nivolumab plus chemotherapy; n = 789; or chemotherapy alone; n = 792). In patients with a PD-L1 CPS greater than 5, they found that Nivolumab with chemotherapy led to substantial improvements in OS and PFS compared to chemotherapy alone. The patients with a PD-L1 CPS > 1 and all randomly assigned people showed a significant improvement in OS as well as a benefit in PFS, according to further data. Of the 782 participants in the nivolumab + chemotherapy group, 462 (59%) and the 767 patients in the chemotherapy alone group, respectively, experienced treatment-related side events. In both groups, the most prevalent any-grade treatment-related side events (25%) were nausea, diarrhea, and peripheral neuropathy. Treatment-related fatalities were determined to be 16 (2%) deaths in the nivolumab plus chemotherapy group and 4 (1%) deaths in the chemotherapy alone group. They proposed that Nivolumab in combination with chemotherapy be the new standard first-line treatment for these patients. In 2021, Shah *et al*[73] conducted a phase 2 open-label, randomized multicenter trial in 141 patients with pretreatment AGC or AGEJC to compare the effectiveness, safety, and pharmacodynamics of Andecaliximab plus Nivolumab *vs* Nivolumab alone. The ORR was 10% with Andecaliximab and Nivolumab and 7% with Nivolumab alone. The addition of Andecaliximab had no effect on response or survival. They concluded that the combination of Andecaliximab and Nivolumab had a positive safety profile but did not increase effectiveness in these individuals when compared to Nivolumab alone. Positive HER-2, greater TMB or GRB7, and lower TGF-β1 were all related to better clinical outcomes.

For the purpose of comparing the effectiveness of Nivolumab plus oxaliplatin-based chemotherapy *vs* placebo plus oxaliplatin-based chemotherapy as first-line therapy, Kang *et al*[74] conducted a randomized, multicenter, double-blind, placebo-controlled, phase 2/3 trial (ATTRACTION-4) in 724 patients with HER-2-negative, unresectable AGC or AGEJC. They discovered that the median PFS in the Nivolumab plus chemotherapy group was 10.45 mo and 8.34 mo in the placebo plus chemotherapy group. After a 26.6-mo follow-up, the median OS in the Nivolumab plus chemotherapy group was 17.45 mo and 17.15 mo in the placebo plus chemotherapy group. They hypothesized that Nivolumab in combination with oxaliplatin-based chemotherapy enhanced PFS but not OS in these patients. Shitara *et al*[75] conducted a randomized study to compare Nivolumab plus chemotherapy *vs* chemotherapy alone (n = 1581), while CheckMate-649 provided the first findings comparing Nivolumab plus Ipilimumab *vs* chemotherapy alone (n = 813). They observed that, after a 24-mo follow-up, Nivolumab with chemotherapy outperformed treatment in patients with PD-L1 CPS ≥ 5; the median OS was 14.4 mo *vs* 11.1 mo, respectively. The risk of mortality was reduced by 30%, and the proportion of patients living at 24 mo was 31% *vs* 19%, respectively. In patients with PD-L1 CPS ≥ 5 or all randomized participants, PFS and ORR were not improved by Nivolumab with Ipilimumab *vs* chemotherapy. However, in both PD-L1 CPS ≥ 5 and all randomized individuals, responses were more sustained with Nivolumab plus Ipilimumab *vs* chemotherapy (median DOR, 13.2 *vs* 6.9 mo). They proposed that the long-term clinically relevant OS and PFS benefits, enhanced and persistent responses, sustained health-related quality of life, and tolerable safety profile of Nivolumab with chemotherapy imply a favorable benefit-risk profile. These findings support the use of this regimen as a conventional first-line therapy in previously untreated AGC or AGEJC patients.

Avelumab

Bang *et al*[76] conducted a randomized, phase 3 JAVELIN Gastric 300 study in 371 patients with AGC or AGEJC in 2018 to assess the contribution of Avelumab to the physician's choice of chemotherapy as third-line treatment. The trial's primary end objectives of increasing OS (4.6 *vs* 5.0 mo) or secondary end criteria of PFS (1.4 *vs* 2.7 mo) or ORR (2.2% *vs* 4.3%) in the Avelumab *vs* chemotherapy groups, respectively, were not met. They claimed that treating these patients with single-agent Avelumab in the third-line scenario did not enhance OS or PFS when compared to chemotherapy. Avelumab, on the other hand, had a more controllable safety profile than chemotherapy. Moehler *et al*[77] conducted a worldwide, open-label, phase 3 JAVELIN Gastric 100 study in 499 AGC or AGEJC patients in 2021 to examine Avelumab maintenance treatment following first-line induction chemotherapy. They found that with Avelumab against chemotherapy, the median OS was 10.4 mo *vs* 10.9 mo, and the 24-mo OS rate was 22.1% *vs* 15.5% with no significant difference. They stated that in patients with AGC or AGEJC in general or in a predetermined PD-L1-positive population, JAVELIN Gastric 100 did not offer a superior OS with Avelumab maintenance *vs* continuing chemotherapy.

Durvalumab

Durvalumab is a human IgG1 monoclonal antibody with a high affinity for blocking PD-L1 binding to CD80 and PD-1. According to available data, 10 mg/kg of single-agent Durvalumab administered intravenously every two weeks for 12 mo showed prospective therapeutic efficacy in gastroesophageal malignancies[78,79]. Kwon *et al*[80] conducted a phase 2 open-label, single-center, non-randomized research study in 31 patients with AGC to assess the effectiveness and safety of Ceralasertib in conjunction with Durvalumab. The ORR, DCR, median PFS, and OS were reported to be 22.6%, 58.1%, 3.0 mo, and 6.7 mo, respectively. Common adverse effects were treatable by adjusting the dosage. In comparison to patients with intact ataxia telangiectasia mutated (ATM) and low sig. HRD (5.60 mo *vs* 1.65 mo), a subset of patients with ATM expression loss and/or a large proportion of mutational signature owing to homologous repair failure (high sig. HRD) had significantly higher PFS. They proposed that Ceralasertib in combination with Durvalumab had potential anticancer efficacy, with long-term responses in patients with refractory AGC.

ACTIIVE IMMUNOTHERAPY

GC vaccine

The ability of cancer vaccines to activate and boost anticancer immune responses, which are predominantly mediated by T cells that detect tumor-associated antigens, gives them therapeutic potential. The ideal vaccine should be easy to give, safe, cheap to produce, and able to induce a memory response that provides long-lasting immunity. By stimulating NK cells, B lymphocytes, and naïve and memory T cells, DCs, APCs, play a crucial role in orchestrating and coordinating antitumor immune responses[81-83]. For presentation to cytotoxic CD8+ T cells or to CD4+ helper T lymphocytes, tumor antigens are loaded by DCs as short peptides onto MHC class I or II molecules. These functional features prompted the development of several ways to use DCs in cancer immunotherapy. Despite these presumptions, the low *in vivo* viability of DC-based vaccines prevents their widespread use in clinical settings. A larger number of DCs infiltrating the tumor in GC patients was found to correspond with reduced lymph node metastases and lymphatic invasion, as well as better 5-year survival rates[84,85]. The results suggest that synthetic tumor peptides, synthetic tumor antigen mRNA, lysates, vesicles, and inactivated tumor cells can all be used as DC vaccine-loaded antigens. Potential GC vaccine antigens include the melanoma-associated antigen A3, HER-2 (p369) peptide, gastin-17 diphtheria toxoid, URLC10 or VEGFR1 epitope, and heat shock protein GP96[86]. Regrettably, there haven't been many beneficial findings using DC vaccinations in the treatment of GC. Only three patients with GC participated in the phase 1/2 clinical study, and only one of them was effective, despite showing that a Wilms tumor 1-targeted DC vaccine might be utilized to treat advanced cancer, including GC. In order to increase the effectiveness of GC vaccines, techniques to target numerous antigens have been investigated[87]. DC vaccines can be used with chemotherapy, radiation, and ICIs to increase efficacy. DC immunizations are safe for AGC patients since their toxicity and side effects include fever, flu-like symptoms, and local reactions at the injection site. DC-cytokine induced killer cell (DC-CIK) therapy is another method of using DCs to treat tumors. DC-CIK, coupled with chemotherapy, was proven in clinical trials to be effective and well tolerated in the treatment of AGC. According to a meta-analysis, patients who get DC-CIK and chemotherapy together after GC surgery have dramatically improved OS, DFS, and T cell responses. Additionally, DC-CIK combined with S-1 and cisplatin showed good PFS and OS in the treatment of AGC, and the combination therapy was safe and well tolerated in terms of toxicity[88,89]. Tumor-infiltrating DCs are linked with a better prognosis in GC; however, the tumor microenvironment contains only a few mature DCs. Therefore, DC vaccines and DC-CIK are ineffective against cancer when used as a single therapy; therefore, it is essential to determine the reasons for the ineffectiveness or combine them with other cancer therapies in order to increase the anti-tumor effects[90].

CONCLUSION

Globally, GC incidence is still high, and because early cancer screening is not extensively used and the symptoms are not frequently recognized, the majority of patients are discovered in the middle or late stages of the disease. After several years of effort, the OS has not dramatically improved the treatment of AGC. Immunotherapy, on the other hand, has given these patients hope. There are several ways to target immune cells to treat tumors, with therapy options targeting T cells having the most substantial effect and the quickest development, as well as showing strong clinical success in various solid tumors, including GC. ICIs were the most important passive immunotherapy in enhancing AGC patients' OS and PFS. These immunotherapies, however, have limitations in the treatment of GC. In certain studies, anti-PD-1/PD-L1 and anti-CTLA-4 antibodies did not improve patients' OS and PFS when compared to chemotherapy. Although encouraging results have been reported in previous clinical studies, the bulk of these populations-which do not make up the majority of AGC patients-have only benefitted from the treatment when their PD-L1 CPS, MSH-H, or TMB scores are high. Although several strategies for targeting immune cells to treat cancer have shown promise in preclinical animal models, they have not been widely used in clinical settings. Because the results of several therapy approaches' ongoing clinical trials have not yet been made public. Another explanation would be that they are only marginally effective, like with the two DC-based cancer treatment methods (vaccination and DC-CIK), which work best in combination with other therapies like chemotherapy. While others acquire primary or secondary medication resistance, the widely used ICIs only work for a portion of tumor patients. The complex microenvironment in which the tumor is located may be the cause of immunotherapy's poor effectiveness. Current immunotherapy only targets one type of cell or a specific target on a specific type of cell, whereas the immunosuppressive environment is made up of multiple cells and multiple targets. The interaction of tumor cells, immune cells, and stromal cells in the tumor microenvironment creates a massive immune suppression network that results in tumor immune escape. More than 10 distinct categories of immunosuppressive receptors expressed on T cells have been identified, and there are still other inhibitory receptors that have not yet been found. The development of therapy modalities with several targets and cells may be the following development path. On the basis of this, multi-target combination approaches for tumor therapy have been developed. Examples include the pairing of PD-1/PD-L1 inhibitors with CTLA-4 inhibitors and PD-1 inhibitors with anti-LAG-3. Moreover, therapeutic tactics targeting immune cells have shown promising outcomes when combined with other therapies, such as chemotherapy medications. As a result, anti-tumor treatment targeting immune cells has a long way to go to achieve synergy and detoxification.

FOOTNOTES

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Country/Territory of origin: Thailand

ORCID number: Wattana Leowattana 0000-0003-4257-2480; Tawithep Leowattana 0000-0003-2316-3585.

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Case Control Study

Characterization and risk factors for unexplained female infertility in Sudan: A case-control study

Abdullah Abdulslam Abdullah, Musa Ahmed, Adesina Oladokun

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Abdullah Abdulslam Abdullah, Musa Ahmed, Reproductive Health Sciences Program, Pan African University Life and Earth Sciences Institute, University of Ibadan, Ibadan 119, Oyo state, Nigeria

Abdullah Abdulslam Abdullah, Musa Ahmed, Adesina Oladokun, Department of Obstetrics and Gynecology, College of Medicine, University of Ibadan, Ibadan 119, Oyo state, Nigeria

Abdullah Abdulslam Abdullah, Department of Biomedical Sciences, Faculty of Veterinary Sciences, University of Gadarif, Al-Gadarif 32211, Sudan

Musa Ahmed, Department of Veterinary Surgery, Faculty of Veterinary Medicine, AL-Salam University, Al-fula 120, West Kordofan, Sudan

Corresponding author: Abdullah Abdulslam Abdullah, MSc, Academic Research, Reproductive Health Sciences Program, Pan African University Life and Earth Sciences Institute, University of Ibadan, Ajibode Road, 200132, Ibadan 119, Oyo state, Nigeria. bahlol32029@gmail.com

Abstract

BACKGROUND

Female infertility with unknown causes (unexplained) remains one of the mysteries in the reproductive health field, where the diagnostic evidence is still weak and the proposed treatments still work with unknown methods. However, several studies have proposed some possible causes and risk factors for unexplained female infertility.

AIM

To characterize and identify factors associated with unexplained infertility in Sudanese women.

METHODS

A matched (age and body mass index) case-control study was conducted from March 2021 to February 2022. The study samples were 210 women with unexplained infertility (UI) and 190 fertile women of reproductive age who were attending the maternity hospitals and fertility clinics in Khartoum, Sudan. The risk factors of unexplained infertility were identified using a structured, pre-tested questionnaire containing information on socio-demographic variables, anthropometrics, clinical diagnosis of infertility, behavioral factors, physical activity assessment, diversity, and consumption of different food groups by the

study participants.

RESULTS

The results showed a higher proportion of women diagnosed with UI were residents of rural areas than controls (21.4% *vs* 11.1%, $P < 0.05$), and previous miscarriages and/or abortions were more common in fertile women compared with infertile women (13.16% *vs* 5.71%, $P < 0.05$). Additionally, infertile women had a significantly ($P < 0.05$) higher proportion of family history of infertility (explained and unexplained) compared with controls. Finally, after controlling for the effects of potentially confounding variables using multivariable logistic regression analysis, only marital status, family history of infertility, use of modern contraceptives, smoking, caffeine consumption, physical activity level, meals consumed, other vitamin-A-rich fruits and vegetables, and other vegetables were found to be significant ($P < 0.05$) factors associated with unexplained infertility among Sudanese women.

CONCLUSION

Married women with a family history of infertility who smoke and consume a high amount of caffeine, who live a sedentary lifestyle, and who consume more than two meals free of vitamin-A-rich fruits and/or vegetables and/or other vegetables per day are at the highest risk of developing unexplained infertility.

Key Words: Unexplained infertility; Sudanese women; Risk factors; Dietary diversity; Physical activity level

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Core Tip: A matched (age and body mass index) case-control study was conducted to characterize and identify factors associated with unexplained infertility in Sudanese women. Four hundred women of reproductive age attending the maternity hospitals and fertility clinics in Khartoum, Sudan, were included in this study. The result showed that married women with a family history of infertility who smoke and consume a high amount of caffeine, who live a sedentary lifestyle, and who consume more than two meals free of vitamin-A-rich fruits and/or vegetables and/or other vegetables per day are at the highest risk of developing unexplained infertility.

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INTRODUCTION

Unexplained infertility (UI) refers to a diagnosis (or lack of diagnosis) made in infertile couples in whom all the standard approved infertility investigations, such as tests of ovulation, tubal patency, and semen analysis, are normal[1,2]. A "diagnosis of unexplained infertility" may be very frustrating for an infertile couple and is often interpreted as if there is no explanation for the cause of infertility, so there is no effective treatment"[3]. Importantly, the prognosis of UI is worse when the duration of infertility exceeds 3 years and the female partner is > 35 years of age[4].

Furthermore, the unexplained infertility estimates vary, and the likelihood that all such tests for an infertile couple are normal (*i.e.*, that the couple has unexplained infertility) is approximately between 10% and 37% for all couples worldwide, and usually, female-related causes are responsible for 50% of it [4-9].

In Africa, the unexplained infertility prevalence is higher than that of other continents and ranges from 10%–37%[6,7]. In Sudan, a systematic review and meta-analysis found that the pooled prevalence of unexplained infertility in the Sudanese population is 17% with a confidence interval from 10% to 24% [6].

The high prevalence of unexplained infertility worldwide, especially in limited-resource countries like Sudan, implies that the current assessment of the human reproductive system is far from perfect. Nevertheless, significant improvements in diagnostic tools and assisted reproductive technologies have led to the finding of many causes of infertility that in the past had only been suspected, but, up until now, some causes of female infertility are still unknown. Subsequently, there is a need to search for more answers to the causes of female infertility to create better treatment options for patients.

Unexplained female infertility is considered one of the hot topics in reproductive medicine, and has been studied extensively; nevertheless, the studies in the area of diagnosis and treatment of unexplained female infertility didn't give concrete answers to the must-answer questions about the diagnosis and treatment of unexplained female infertility, whereby the diagnostic evidence is still weak and the proposed treatments still work with unknown methods. However, these studies have proposed some possible causes and risk factors for unexplained female infertility.

Studies conducted in low and low-middle-income countries showed that sociodemographic characteristics such as high body mass index (BMI), high waist-to-hip ratio (WHR), age older than 35 years, late marriage age, residence in rural and agricultural areas, low educational attainment, and unemployment were found to be significant risk factors for female infertility[8,10-16]. Furthermore, three systematic review and meta-analysis studies found a strong association between smoking and female infertility, whereby the pooled estimation showed that smoking women have a 1.8 times higher risk of developing infertility than women who do not smoke. Also, the same studies found a significant relationship between female infertility and alcohol consumption[10,17,18].

Additionally, other studies showed that high caffeine consumption was significantly associated with female infertility[12,19]. Meanwhile, several studies have found a negative significant association between increased physical activity level and unexplained female infertility[20], with sedentary women 3.61 times more likely to have unexplained infertility than moderately or very active women[19,21,22].

Furthermore, studies conducted in Iran and France found that women with a family history of infertility were 3.88 times more likely to develop unexplained infertility than women who did not have any close relatives who had experienced infertility previously[21,23]. This conclusion leads us to consider genetic factors as causes of unexplained infertility.

The previous history of abortion in women was one of the significant risk factors related to female infertility, whereby women with a previous history of abortion were more likely to have infertility in comparison to women without any previous history of abortion, with odds ratios of 9.33, 1.63, and 2.381, respectively, for Nigerian citizens[24], southeast Iranian citizens[23], and central Iranian citizens [25]. Some reports listed the use of contraceptives as a possible risk factor for female infertility[23,24]; in contrast, other reports found opposite results[13,26]. Therefore, there is still a need to find the association between the use of contraceptives and unexplained female infertility.

It is well known that nutritional status and the selection of food groups and supplements are crucial determinants of normal reproductive function. World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) described ten essential food groups for normal reproductive function in women of reproductive age[27,28]. Interestingly, in the available literature, there is no study about the effect of nutrition and diet diversity on unexplained female infertility, and the available evidence is only reported from cases of explained female infertility.

An 8-year prospective cohort study conducted by Chavarro *et al*[29], about the association between dietary diversity, eating habits, and the risk of female infertility, laid the basic foundation of what is now called a "fertility diet," which is a diet intended to increase the chances of women in the reproductive age of ovulation through changes in eating patterns and physical activity, whereby women with a high fertility diet score tend to have long menstrual cycles, normal reproductive hormone levels, and normal ovarian folliculogenesis. In addition, the findings of this study showed that the high fertility diet score was characterized by a lower intake of trans fat with a greater intake of monounsaturated fat; a lower intake of animal protein with a greater vegetable protein intake; a higher intake of high-fiber, low-glycemic carbohydrates; a greater preference for high-fat dairy products; a higher plant-based iron intake; and a higher frequency of multivitamin use[29]. Similarly, a nested case-control study in a Spanish cohort of 2154 female university graduates found that women with the highest adherence to the Mediterranean diet (characterized by high consumption of fruits and vegetables, fish, and poultry, low-fat products, and olive oil, which provides monounsaturated fat, and low consumption of red meat and sweets) are less likely to experience fertility difficulties[30].

Based on an extensive review of the existing literature about the risk factors of unexplained female infertility, the current study investigates sociodemographics, clinical history, dietary diversity, and consumption of different food groups as distinguishing factors associated with unexplained female infertility. More studies are needed to keep track of localized trends in infertility for better clinical management and research strategy as there is more variety and trends in unexplained infertility issues globally.

The present study highlights the characterization of unexplained infertility among Sudanese women and identifies factors associated with unexplained female infertility. The study outcome will contribute to the existing limited knowledge of unexplained female infertility and also have great significance for infertility program coordinators and policymakers as they design and implement effective strategies for preventing and managing female infertility.

MATERIALS AND METHODS

Study design and period

The objectives of this study were achieved using a prospectively matched age and BMI case-control design. The study was carried out during the period from March 2021 to February 2022.

Study population, setting, and area

Prof. El-Sir Abo Elhassan Fertility Center, Omdurman Maternity Hospital, Nile Fertility Center, Khartoum Reproductive Health Care Center, Sudan Assisted Reproductive Clinic, University of Khartoum Fertility Center, Saad Abualila Teaching Hospital, Banoun Fertility Center, and Hawwa Center for Fertility were all used to collect study samples. Those centers were purposefully selected because they are the largest centers providing assisted conception and modern antenatal care in Sudan.

Study participants' selection and eligibility criteria

The current study targeted 420 Sudanese women between the ages of 18 and 44. The case group included 210 women with unexplained infertility; the women were chosen using a systematic random sampling technique from patients who had tried but were unable to conceive for at least a year (maintain regular unprotected vaginal sexual intercourse with their partner at least twice a week for a year); cases should also have regular menstruation, a normal ovarian reserve test result, open fallopian tubes, and a normal uterus cavity size according to the hysterosalpingography[1].

In addition, their couples should have a normal spermiogram "with a concentration of at least 15 million sperm per milliliter, a motility value over 70 percent, and morphology of more than 4 percent with normal forms"[31]; the control group consisted of 210 fertile, nonpregnant women who had at least one healthy 2-year-old child (end of lactation).

Women were excluded from this study if they had any of the following illnesses: Hypertension, diabetes, endocrine disorders, autoimmune or immunocompromised conditions, a history of genetic disease, or sexually transmitted infections. In addition to that, women were also excluded if they were under the effect of anti-inflammatory medicines or if they were under the effect of hormonal contraception within the last 6 mo. Finally, any women who refused to sign the informed consent form or withdrew during the study were excluded.

Data collection procedures and instruments

To assess unexplained female infertility risk factors, a structured pre-tested questionnaire containing information on socio-demographic variables, anthropometrics, clinical diagnosis of infertility, behavioral factors, physical activity assessment, dietary diversity, and consumption of different food groups by study participants was used.

The questionnaire was prepared by reviewing several relevant published articles[19,32-34] and adopting standardized data collection tools[22,27]. Primarily, the questionnaire was prepared in the English language and translated into the country's main local language (Arabic). The consistency of the questionnaire was checked in different phases: First, by translating it back to English and reviewing it with a non-affiliated researcher with good knowledge of both languages; after that, the final form of the questionnaire was tested for consistency using internal consistency (Cronbach's alpha of 0.73); and then it was pretested by distributing it among 30 women not participating in the actual study but living in a similar setting.

After passing all quality check-ups, the questionnaire was filled out with the help of 10 research assistants who are experts in reproductive health (one in each previously mentioned healthcare facility) and who were trained on questionnaire administration skills relevant to this study by the primary investigator and charged with seeking signed informed consent from the study participants. The completeness of the data was checked each day at the end of data collection. Incomplete data was traced back and edited accordingly. The follow-up of study participants was done by tracking information (address, phone number of the participant as well as of relatives and close friends) and making periodic contact (reminders, updates) to minimize loss of follow-up, and the overall follow-up and data collection processes were coordinated and supervised by the research assistants' and principal investigator's.

Operational definition of the variables

Unexplained infertility was defined as a type of infertility that occurs when standard-approved infertility tests have not found a clear cause for the couple's inability to achieve pregnancy[35].

BMI is defined as an estimation of human body fat based on height and weight. BMI is expressed in kg/m^2, resulting from dividing body mass in kilograms by height in meters[36,37].

The WHR was defined as an estimation of fat stored around the waist and hips. The waist-hip ratio was calculated by dividing the waist measurement by the hip measurement.

Caffeine consumption: Caffeine is a central nervous system stimulant. It is used as a cognitive enhancer, increasing alertness and attentional performance. In this study, caffeine consumption was only from liquid sources of stimulants. It is used as a cognitive enhancer, increasing alertness and

attentional performance. In this study, caffeine consumption was only from liquid sources. It is used as a cognitive enhancer, increasing alertness and attentional performance. In this study, caffeine consumption was only from liquid sources (tea, coffee, sodas, and energy drinks) and was estimated based on the number of drunk cups in 24 h, whereby no consumption was considered if the woman didn't "drink any cups of caffeine," low consumption if the woman "drank a cup or two a day," moderate consumption if the woman "drunk 3 or 4 cups in 24 h," and high consumption if the woman "drunk more than 4 cups in 24 h".

Physical activity level is a way to express a person's daily physical activity as a number and is used to estimate a person's total energy expenditure. The physical activity level was estimated using a list of the physical activities a woman performs within a 24-h period and the amount of time spent on each activity. The following shows physical activity levels for several lifestyles: (Inactive = hospital patient with limited physical mobility; sedentary = office worker getting little or no exercise; moderately active = moderate physical activity at work or leisure; very active = considerable physical activity at work, *e.g.*, agricultural worker (non-mechanized) or office workers who take at least moderate exercise for two or more hours per day; and extremely active = professional athlete or sports person, *e.g.*, football player) [38]. Dietary diversity is a qualitative measure of food consumption that reflects women's access to a variety of foods, including grains, nuts, dairy products, meat, eggs, fruits, and vegetables. Dietary diversity was determined based on a 7-d recall method. For each group, no consumption was considered "no servings per week," low consumption "1 or 2 servings per week," moderate consumption "3 servings per week," and high consumption "more than 3 servings per week." Servings per week were defined considering the consumption considered adequate in the African and Arabic diets[27,39].

Data management and statistical analysis plan

Data collected from this study were sorted and recorded in Microsoft Excel 2016, cleaned, and then transferred to STATA software, version 16.0 (Stata Corp LLC, 77845 Texas, United States), and Jeffrey's Amazing Statistics Program, version 0.16.4.0 (JASP), for analysis. To characterize the study population, descriptive statistics, frequencies, and percentages for categorical data and summary statistics (mean standard deviation (SD) with a 95% confidence interval (CI) for continuous data normally distributed and median and interquartile range for continuous data not normally distributed) were used. In addition, tables and figures were used for data presentation. The normal distribution of the study variables (univariate, pairwise, and multivariate[40]) was performed using the Shapiro-Wilk test, and the data were considered normally distributed if the p-value was greater than 0.05.

The association between the categorical variables in the study population was checked using the chi-square test at the statistically significant level of $P = 0.05$. Meanwhile, this association was estimated using Cramer's V statistic effect size test, according to which it was classified, according to Kim[41], 2017, into small, medium, and large effect sizes.

A binary logistic regression analysis (bi-variable and multivariable) was carried out to identify the independent predictors of unexplained infertility. All independent variables with a P value of less than 0.05 in the bivariable logistic regression model were considered candidate variables for the multivariable model. Finally, the relationship was presented using a crude odds ratio and an adjusted odds ratio (AOR) with their corresponding 95% confidence intervals, and a P value of 0.05 or less was considered to be statistically significant.

RESULTS

Anthropometry and socio-demographic characteristics of study participants

The overall response rate in the current study was 210 (100%) among cases and 190 (90.5%) among controls.

Finally, the study included 400 women (210 women with UI as case subjects and 190 fertile women as control subjects). The two study groups were matched by age and BMI. The mean age of cases was 28.59 years with a SD of 5.22 years and a CI: of 27.87–29.3 years, while it was 28.44 years for controls (SD 4.95 years and a CI: of 27.73–29.15 years). The mean BMI values were 24.67 (SD 4.08 and CI: 24.11–25.22) and 24.41 (SD 4.38 and CI: 23.78–25.03) for the cases and controls, respectively. Besides, the two groups had almost the same mean value of the WHR [0.844 (SD 0.108 and CI: 0.829–0.859) for the cases and 0.837 (SD 0.114 and CI: 0.821–0.854) for the controls].

In addition to the above-mentioned anthropometry parameters, other sociodemographic characteristics of study participants, such as place of residence, marital status, education status, religious affiliation, and occupation, were checked. The only place of residence and marital status showed a statistically significant difference between the two study groups (Table 1). Wherein more women diagnosed with UI lived in rural areas than in urban areas (21.4% *vs* 11.1%, $P < 0.05$). Furthermore, when compared to controls, the vast majority of cases (99.1% *vs* 89.5%, $P < 0.05$) were married.

Meanwhile, the estimation of the effect sizes (Cramer's V) indicated a small association between female unexplained infertility (age, BMI, WHR, residence, education status, and religious affiliation) and a medium association between female unexplained infertility and (marital status and occupation).

Table 1 Characterization of selected anthropometry and socio-demographic variables of study participants, *n* (%)

Variable	Categories	Cases (*n* = 210), N%	Control (*n* = 190), N%	Effect size Cramer's V	*P* value
Age group (yr)	18-24	49 (23.3)	40(21.05)	0.08[3]	0.61
	25-29	87 (41.4)	91(47.9)		
	30-34	44 (21)	35(18.4)		
	35-39	23 (11)	21(11.05)		
	40-44	7 (3.3)	3(1.6)		
	mean ± SD	28.59 ± 5.22	28.44 ± 4.95	NA	NA
BMI (kg/m^2)	Underweight	1 (0.5)	4 (2.1)	0.07[3]	0.53
	Normal weight	127 (60.5)	111 (58.4)		
	Overweight	59 (28.1)	54 (28.4)		
	Obesity	23 (10.9)	21 (11.1)		
	mean ± SD	24.67 ± 4.08	24.41 ± 4.38	NA	NA
WHR	Less than 0.75	46 (22)	54 (28.4)	0.11[3]	0.08
	075-0.84	53 (25.2)	35 (18.4)		
	0.85-0.90	45 (21.4)	41 (21.6)		
	Greater than 90	66 (31.4)	60 (31.6)		
	mean ± SD	0.844 ± 0.108	0.837 ± 0.114	NA	NA
Residence	Rural	45 (21.4)	21 (11.1)	0.14[3]	**0.005**
	Urban	165 (78.6)	169 (88.9)		
Marital status	Married	208 (99.1)	170 (89.5)	0.21[4]	**0.001**
	Divorced[1]	2 (0.9)	13 (6.8)		
	Widow	0 (0)	7 (3.7)		
Education status	Diploma or above	89 (42.4)	85 (44.7)	0.05[3]	0.85
	Secondary edu	72 (34.3)	65 (34.2)		
	Primary edu	39 (18.6)	34 (17.9)		
	No formal edu[2]	10 (4.8)	6 (3.2)		
Religious affiliation	Muslim	198 (94.3)	184 (96.8)	0.06[3]	0.22
	Christian	12 (5.7)	6 (3.2)		
Occupation	Farmer	9 (4.3)	2 (1.1)	0.16[4]	0.06
	Governmental	34 (16.2)	29 (15.3)		
	Housewife	132 (62.9)	110 (57.9)		
	Private business	28 (13.3)	34 (17.9)		
	NGOs	1 (0.5)	6 (3.2)		
	Student	6 (2.9)	9 (4.7)		

[1]Information about, age, body mass index, and waist-to-hip ratio were reused from reference[81].
[2]Divorced women must have remained in regular sex in the last year intending to conceive.
[3]This includes the type of religious education (Khalwa). In the last column to the right, bold values indicate a significant level at $P < 0.05$. Cramer's V effect size was classified according to Kim[41], 2017 as small effect size.
[4]Medium effect size. Differences between groups were compared with chi-square test. Estimated effect sizes were calculated using Cramer's V statistic which is expressed as mean. Body mass index and waist-to-hip ratio status were follows World Health Organization and Centers for Disease Control and Prevention's classifications[36,37]. BMI: Body mass index; *n*: Sample size; NA: Not available; NGOs: Non-governmental organizations; SD: Standard deviation; WHR: Waist–hip ratio.

History and clinical characteristics of study participants

Table 2 shows the detailed history and clinical characteristics of the study participants. In this study, the median duration of unexplained infertility in cases was 3 years, with a range of 1–15 years and an interquartile range of 3.75, compared to the controls, who had a median of 4 for marriage duration, with a range of 3–20 years and an interquartile range of 2. These results were not compared since they were dissimilar.

Interestingly, fertile women had more previous miscarriages or abortions than infertile women (13.16% *vs* 5.71%, $P < 0.05$).

Furthermore, the current study found that infertile women had a higher proportion of infertility in their families than fertile women (28.1% *vs* 6.3%, $P < 0.05$). Similarly, the proportion of unexplained infertility in infertile women's families was higher than in controls (13.8% *vs* 1.6%, $P < 0.05$).

However, other clinical history variables did not show any significant association ($P > 0.05$) with unexplained female infertility and were reported as follows: The majority of study participants did not use contraceptives (64.8% for cases *vs* 62.6% for controls), none of the study participants have ever consumed alcohol, and the majority of them do not smoke (86.2% for cases and 91.6% for controls). Furthermore, the caffeine consumption patterns were almost the same in the two groups, whereby no consumption was 3.8% for cases *vs* 3.2% for controls, low consumption was 32.4% for cases *vs* 36.3% for controls, moderate consumption was 31.4% for cases *vs* 26.8% for controls, and high consumption was 32.4% for cases *vs* 33.7% for controls.

Moreover, the three (sedentary, moderately active, and very active) physical activity levels were at the same levels in the two groups, and the majority of the study participants had a sedentary lifestyle (50.5% for cases *vs* 48.1% for controls).

Finally, as can be seen in table 3, the estimation of the effect sizes (Cramer's V) indicated a small association between female unexplained infertility and (previous miscarriage or abortion, family history of infertility, family history of unexplained infertility, use of contraceptives, smoking, caffeine consumption, and physical activity levels) and a medium association between female unexplained infertility and the number of miscarriages or abortions.

Dietary diversity and consumption of different food groups by the study participants

This study examined the consumption patterns of the essential ten food groups described by the WHO and FAO for women of reproductive age and the number of daily meals based on a 7-d recall method. The results found that there was no significant association ($P > 0.05$) between unexplained infertility and the consumption patterns for each of the ten essential food groups and the number of daily meals consumed by the study participants.

Additionally, the estimation of the effect sizes (Cramer's V) of these associations was small for all studied variables.

With an in-depth look at the general features of these findings, we can conclude that almost half of the Sudanese women used to consume 3 meals per day (42.4% for cases *vs* 45.3% for controls). Besides, regarding the consumption pattern of specific food groups, grains, and pulses were the most consumed food groups by study participants, whereas nuts, meat (including red meat, poultry, and fish), and eggs were the least consumed food groups by both study groups equally. The fully detailed consumption patterns of different food groups by the study participants are described in Table 3 and Figure 1.

Factors associated with unexplained female infertility in the study participants

To identify the unexplained infertility predictor factors among Sudanese women, a binary logistic regression was used. The results found that from all the variables tested using the bi-variable binary logistic regression, only the place of residence, marital status, previous miscarriage or abortion, family history of infertility, family history of unexplained infertility, use of modern contraceptives, smoking, caffeine consumption, physical activity level, meals consumed, dairy, dark green leafy vegetables, other vitamin A-rich fruits and vegetables, and other vegetables were found to have significant associations with unexplained female infertility.

However, after controlling for the effects of potentially confounding variables using multivariable logistic regression analysis, only marital status, family history of infertility, use of modern contraceptives, smoking, caffeine consumption, physical activity level, meals consumed, other vitamin-A-rich fruits and vegetables, and other vegetables were found to be significant factors associated with unexplained infertility among Sudanese women. The variables eligible for the multivariable logistic regression model are described in Table 4 and Figure 2.

In the current study, married women were 0.073 times more likely to have unexplained female infertility in comparison to divorced and widowed women (AOR: 0.073, 95%CI: -4.431–0.803). Another vital factor significantly associated with unexplained female infertility was the family history of infertility, whereby any woman who has a history of infertility in her family was 3.257 times more likely to have unexplained female infertility compared with other women who don't have any family history of infertility (AOR 3.257, 95%CI: 0.175-2.186).

Meanwhile, the family history of unexplained infertility didn't show any significant associations with unexplained female infertility in the study participants. Women who did not use any contraceptives had

Table 2 History and clinical characteristics of study participants, *n* (%)

Variables	Categories	Cases (*n* = 210), N%	Controls (*n* = 190), N%	Effect size Cramer's V	*P* value
Duration of infertility/marriage duration (yr)		Median = 3; Range = 1-15; IQR (3.75)	Median = 4; Range = 3-20; IQR (2)	NC	NC
Previous miscarriage or abortion	Yes	12 (5.71)	25 (13.16)	0.13[3]	**0.02**
	No	198 (94.29)	165 (86.84)		
Number of miscarriages or abortions	Never	198 (94.29)	165 (86.84)	0.16[4]	0.08
	1	6 (2.86)	13 (6.84)		
	2	4 (1.9)	6 (3.16)		
	3	1 (0.48)	4 (2.1)		
	4	0 (0)	2 (1.05)		
	5	1 (0.48)	0 (0)		
Family history of infertility	Yes	59 (28.1)	12 (6.3)	0.29[3]	**0.001**
	No	151 (71.9)	178 (93.7)		
Family history of Unexplained infertility	Yes	29 (13.8)	3 (1.6)	0.23[3]	**0.001**
	No	181 (86.2)	187 (98.4)		
Use of modern contraceptives/yr	Never	136 (64.8)	119 (62.6)	0.09[3]	0.28
	0-3 yr	53 (25.2)	60 (31.6)		
	4-6 yr	16 (7.6)	9 (4.7)		
	More than 6 yr	5 (2.4)	2 (1.1)		
	Not in use now	0 (0)	0 (0)		
Alcohol consumption	Yes	0 (0)	0 (0)	NE	NE
	Not at all	210 (100)	190 (100)		
	Stopped taking	0 (0)	0 (0)		
Smoking[1]	Not at all	181 (86.2)	174 (91.6)	0.09[3]	0.54
	< 1 yr	1 (0.48)	1 (0.53)		
	1-2 yr	6 (2.9)	3 (1.6)		
	2-3 yr	9 (4.3)	5 (2.6)		
	> 4	13 (6.2)	7 (3.7)		
Caffeine consumption (cup/d)[2]	No consume	8 (3.8)	6 (3.2)	0.06[3]	0.72
	Low consume	68 (32.4)	69 (36.3)		
	Moderate consume	66 (31.4)	51 (26.8)		
	High consume	68 (32.4)	64 (33.7)		
Physical activity level	Very active	43 (22.6)	38 (18.1)	0.08[3]	0.26
	Moderately active	51 (26.9)	71 (33.8)		
	Sedentary	96 (50.5)	101 (48.1)		

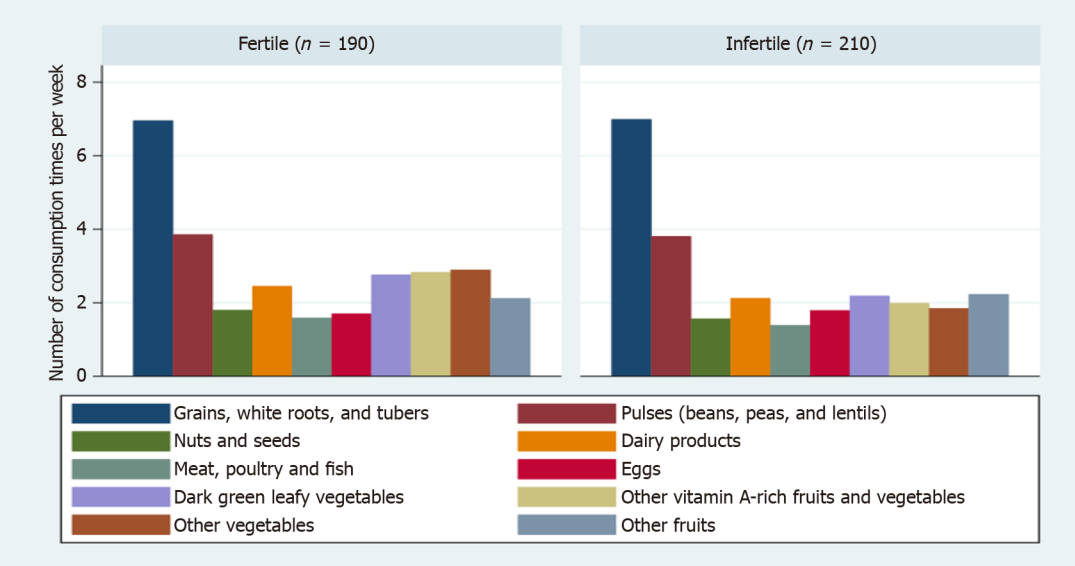
[1]The reported tobacco smoking in this study was in two forms [cigarettes, and shisha (water pipe)].
[2]Caffeine consumption was based only on liquid sources of caffeine (Tea, coffee, Sodas, and Energy Drinks).
[3]In the last column to the right, bold values indicate a significant level at $P < 0.05$. Cramer's V effect size was classified according to Kim (2017)(41) as small effect size.
[4]Medium effect size.
Differences between groups were compared with chi-square test. Estimated effect sizes were calculated using Cramer's V statistic which is expressed as mean. Physical activity level follows the WHO classification[38]. IQR: Interquartile range; *n*: Sample size; NC: Not Comparable due to dissimilar data; NE: Not evaluated in STATA due to zero value in one of the study groups.

Table 3 Frequency (Percentage) of dietary diversity and consumption patterns of different food groups by the study participants, *n* (%)

Variables	Categories	Cases (*n* = 210)	Controls (*n* = 190)	Effect size Cramer's V	*P* value
Meals consumed per day	2	50 (23.8)	44 (23.2)	0.04[1]	0.97
	3	89 (42.4)	86 (45.3)		
	4	42 (20)	34 (17.9)		
	5	24 (11.4)	21 (11.1)		
	6	5 (2.4)	5 (2.6)		
Grains, white roots, and tubers	No consume	0 (0)	0 (0)	NE	NE
	Low consume	0 (0)	0 (0)		
	Moderate consume	0 (0)	0 (0)		
	High consume	210 (100)	190 (100)		
Pulses (beans, peas, and lentils)	No consume	7 (3.3)	4 (2.1)	0.09[1]	0.36
	Low consume	27 (12.9)	36 (18.9)		
	Moderate consume	51 (24.3)	43 (22.6)		
	High consume	125 (59.5)	107 (56.3)		
Nuts and seeds	No consume	57 (27.1)	69 (36.3)	0.11[1]	0.18
	Low consume	88 (41.9)	77 (40.5)		
	Moderate consume	37 (17.6)	25 (13.2)		
	High consume	28 (13.3)	19 (10)		
Dairy products	No consume	37 (17.6)	38 (20)	0.09[1]	0.35
	Low consume	89 (42.4)	64 (33.7)		
	Moderate consume	42 (20)	42 (22.1)		
	High consume	42 (20)	46 (24.2)		
Meat, poultry and fish	No consume	74 (35.2)	65 (34.2)	0.09[1]	0.32
	Low consume	90 (42.9)	69 (36.3)		
	Moderate consume	37 (17.6)	43 (22.6)		
	High consume	9 (4.3)	13 (6.8)		
Eggs	No consume	44 (20.9)	49 (25.8)	0.13[1]	0.09
	Low consume	90 (42.9)	94 (49.8)		
	Moderate consume	60 (28.6)	36 (18.9)		
	High consume	16 (7.6)	11 (5.8)		
Dark green leafy vegetables	No consume	16 (7.6)	13 (6.8)	0.02[1]	0.97
	Low consume	101 (48.1)	89 (46.8)		
	Moderate consume	55 (26.2)	53 (27.9)		
	High consume	38 (18.1)	35 (18.4)		
Other vitamin A-rich fruits and vegetables	No consume	25 (11.9)	25 (13.2)	0.08[1]	0.47
	Low consume	87 (41.4)	75 (39.5)		
	Moderate consume	63 (30)	48 (25.3)		
	High consume	35 (16.7)	42 (22.1)		
Other vegetables	No consume	26 (12.4)	33 (17.4)	0.08[1]	0.49
	Low consume	83 (39.5)	66 (34.7)		
	Moderate consume	57 (27.1)	49 (25.8)		
	High consume	44 (20.9)	42 (22.1)		

Other fruits	No consume	28 (13.3)	30 (15.8)	0.04[1]	0.88
	Low consume	94 (44.8)	84 (44.2)		
	Moderate consume	56 (26.7)	46 (24.2)		
	High consume	32 (15.2)	30 (15.8)		

[1]The significance level was set at $P < 0.05$. Cramer's V effect size was classified according to Kim[41], 2017, as small effect size. Differences between groups were compared with chi-square test. Estimated effect sizes were calculated using Cramer's V statistic which is expressed as mean. The four classifications of the consumption levels of the different food groups per week followed FAO classifications[27]. *n*: Sample size; NE: Not evaluated in STATA due to zero value in one of the study groups.



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Figure 1 Food groups consumed by the study participants (*n* = 400). Values are given as the mean.

significantly higher odds of developing unexplained female infertility compared with those who used them for any period of time (AOR 0.475, 95%CI: -0.987-0.502). In addition, smoking women had a significantly higher risk of developing unexplained female infertility than non-smoking women (AOR 1.276, 95%CI: 0.010–0.476).

This study also revealed that participants who had a sedentary lifestyle were more likely to have unexplained female infertility in comparison to participants with other lifestyles (AOR 0.423, 95%CI: 1.240–0.481). Furthermore, women who consumed more than two meals per day had a higher probability of developing unexplained infertility (AOR 1.606, 95%CI: -0.169-0.778). Additionally, women who didn't consume vitamin-A-rich fruits and vegetables and other vegetables had higher odds of having unexplained female infertility in comparison to those who consumed these two food groups in any amount. On the contrary, as caffeine consumption increases, the odds of having unexplained female infertility decrease (AOR 0.407, 95%CI: 0.168–0.514) (Table 4 and Figure 2).

DISCUSSION

The purpose of this thesis research was to identify factors associated with unexplained infertility in Sudanese women using the chi-square test to check the association and the logistic regression test to identify the unexplained infertility predictor factors. The results of the current study contribute to the limited knowledge of unexplained female infertility and have great significance for infertility program coordinators and policymakers as they design and implement effective strategies for preventing and managing female infertility. Also, the study data will be used as a baseline for other researchers who want to investigate further findings in this area.

Socio-demographic characteristics of the study participants

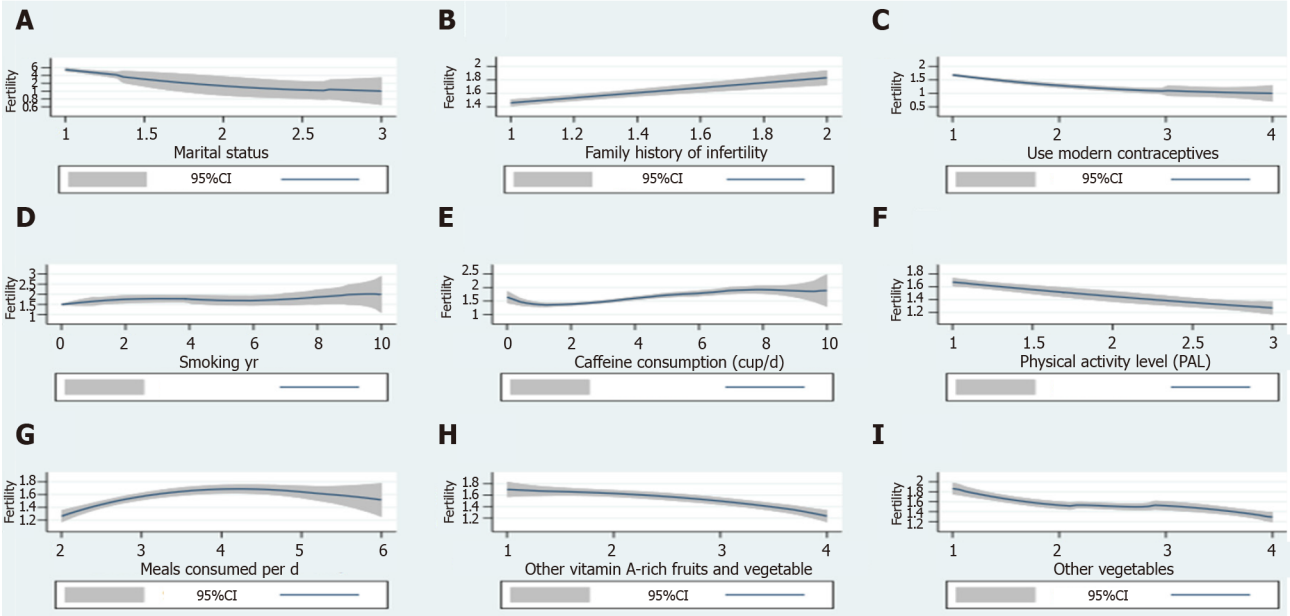
The current study's finding reveals that, from all studied sociodemographic variables, only the place of residence and marital status showed a significant association with unexplained infertility in Sudanese

Table 4 Binary logistic regression analysis of the factors associated with unexplained female infertility in the study participants

Predictors	COR, 95%CI	AOR, 95%CI
Residence		
Urban	Reference	Reference
Rural	2.195 (0.225-1.347)[a]	1.996 (-0.165-1.548)
Marital status		
Married	Reference	Reference
Divorced	0.126 (-3.576-0.571)[a]	0.073 (-4.431-0.803)[a]
Widow	0.419 (-0.922-0.386)[a]	
Previous miscarriage or abortion		
No	Reference	Reference
Yes	0.4 (-1.635 - 0.198)[a]	0.426 (-1.874-0.165)
Family history of infertility		
No	Reference	Reference
Yes	5.796 (1.1 - 2.415)[a]	3.257 (0.175-2.186)[a]
Family history of Unexplained infertility		
No	Reference	Reference
Yes	9.987 (1.095- 3.507)[a]	3.580 (-0.594-3.145)
Use of modern contraceptives/yr		
Never	Reference	Reference
0-3 yr	0.188 (-2.147-1.191)[a]	
4-6 yr	0.052 (-4.446-1.477)[a]	0.475 (-0.987-0.502)[a]
More than 6 yr	0.085 (-0.484-0.823)	
Smoking		
Not at all	Reference	Reference
< 1 yr	1.751 (-0.077-2.434)[a]	
1-2 yr	2.983 (-0.251-2.437)[a]	1.276 (0.010-0.476)[a]
2-3 yr	4.102 (0.117-2.706)[a]	
> 4	3.356 (0.176-2.245)[a]	
Caffeine consumption (cup/d)		
No consume	Reference	Reference
Low consume	0.254 (-2.521-0.219)[a]	
Moderate consume	0.648 (-1.586-0.718)[a]	0.407 (0.168-0.514)[a]
High consume	0.426 (-0.802-1.512)[a]	
Physical activity level (PAL)		
Sedentary	Reference	Reference
Moderately active	0.237 (-1.394- 0.464)[a]	0.423 (-1.240-0.481)[a]
Very active	0.292 (-2.291- 1.145)[a]	
Meals consumed per d		
2	Reference	Reference
3	4.172 (0.876-1.981)[a]	
4	5.293 (1.006-2.326)[a]	1.606 (0.169-0.778)[a]
5	5.286 (0.899-2.432)[a]	

6	2.917 (-0.253-2.394)	
Dairy		
No consume	Reference	Reference
Low consume	1.551 (-0.118-0.997)[a]	
Moderate consume	0.974 (-0.649-0.596)[a]	0.777 (-0.540-0.036)
High consume	0.674 (-1.015-0.226)[a]	
Dark green leafy vegetables		
No consume	Reference	Reference
Low consume	1.014 (-0.780-0.807)[a]	
Moderate consume	0.789 (-1.067-0.593)[a]	0.730 (-0.651-0.021)
High consume	0.346 (-1.948-0.176)[a]	
Other vitamin A-rich fruits and vegetables		
No consume	Reference	Reference
Low consume	0.673 (-1.078-0.287)[a]	
Moderate consume	0.469 (-1.467-0.047)[a]	0.540 (-0.948-0.283)[a]
High consume	0.131 (-2.837-1.232)[a]	
Other vegetables		
No consume	Reference	Reference
Low consume	0.177 (-2.543-0.920)[a]	
Moderate consume	0.169 (-2.614-0.940)[a]	0.466 (-1.087-0.441)[a]
High consume	0.064 (-3.623-1.866)[a]	

[a]Indicates a statistically significant effect at $P < 0.05$. Identification of female unexplained infertility predictors among clinical characteristics was done using a univariate and multivariate logistic regression analysis test. AOR: Adjusted odds ratio; CI: Confidence interval; COR: Crude odds ratio; n: Sample size.



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Figure 2 Variables eligible for the multivariable logistic regression model are described. A: The polynomial association between infertility in Sudanese women, and marital status; B: Family history of infertility; C: Use of modern contraceptives; D: Smoking; E: Caffeine consumption; F: Physical activity level; G: Number of meals consumed per day; H: Other vitamin A-rich fruits and vegetables; I: Other vegetables.

women (Table 2). Specifically, infertility with unknown causes (unexplained) was significantly associated with married women and women residents in rural areas. This might be due to the fact that, in rural areas of Sudan, there are not enough healthcare facilities providing primary healthcare services and consulting in general and specifically for obstetrics and gynecology[42,43]. This can lead to neglecting the initial symptoms of infertility, which exacerbates the infertility conditions and makes it difficult to diagnose and treat them.

Furthermore, sociocultural practices such as believing infertility is a curse and that you should accept it and not seek help from anyone other than God are still prevalent in many rural communities in Sudan. In support of that, in a recent cross-sectional study of infertility management strategies among Sudanese participants, it was discovered that nearly one-half of the participants strongly believed that the best strategy for infertility management is to use Qur'an and Sunna treatments[44]. Such beliefs make it very difficult to diagnose and treat infertility cases in these communities.

All the above-mentioned factors can partially explain the high prevalence of unexplained female infertility among married women in rural areas of Sudan compared with other women in urban areas. This is in line with the findings of other female infertility studies conducted in Iran[13], Pakistan[16], Cameroon[15], the Central African Republic[15], and Chad[15]. These countries have economic settings and religion-oriented communities similar to Sudan; therefore, it is logical to draw similar conclusions.

In this study, 95.5% of the participants were Muslims, so the religious affiliation wasn't comparable, thereby showing no significant association with unexplained infertility in Sudanese women. Meanwhile, other sociodemographic variables, like occupation and education status, were comparable between the two groups; nevertheless, they didn't show any significant association with unexplained infertility in Sudanese women.

This result corroborates other findings reported in Nigeria[24], Iran[13], Pakistan[16], Cameroon[15], the Central African Republic[15], and Chad[15].

On the other hand, in contrast with the findings of the current study, a study conducted on women in Yazd, Iran[23], found a significant association between education status and female infertility, whereby the infertility risk increases with high educational attainment. Interestingly enough, this conclusion went against all the currently available literature.

History and clinical characteristics of the study participants

The present study also investigated the clinical histories of the study participants (Table 3). Overall, the median duration of unexplained infertility among the case group was 3 years, ranging from 1 to 15 years. Furthermore, this study found a significant association between the family history of infertility (explained and unexplained) and unexplained infertility. This may indicate that there may be some genetic factors for unexplained female infertility, but due to the complexity of the reproduction process in females, this possible gene(s) is still unknown. Nevertheless, many studies found that most infertility causes and conditions, such as poor egg quality or low egg reserves and blocked or damaged fallopian tubes, can't be inherited and can happen to anyone, regardless of family history[45,46]. As a result, there is an urgent need for excessive research work to be done in the field of genetic infertility.

Although similar results to the findings of this study describing the association between family history of infertility and female infertility were reported in Iran[13,23,25], and Netherlands[47]. A case-control study conducted in Nigeria in 2020[24], with a smaller sample size, didn't find any significant association between female infertility and a family history of infertility.

It is important to note that, in the present study, the proportion of women with previous miscarriage and/or abortion was higher in fertile women compared with infertile women (13.16% *vs* 5.71%); however, the miscarriage proportion difference between the two groups is not significant.

This finding indicates that having a miscarriage and/or an abortion doesn't affect a woman's ability to get pregnant in the future. A substantial number of studies reached similar conclusions[8,13,48,49]. Meanwhile, this result disagrees with the findings of studies from Nigeria[24], Iran[23], and Germany [50], which found a positive association between secondary infertility and abortion.

In this study, none of the study participants had ever consumed alcohol in any form or amount before. As a result, alcohol consumption among the study participants cannot be compared and is associated with unexplained infertility. The low prevalence of alcohol consumption in Sudanese women [51-53] could be responsible for the zero prevalence of alcohol consumption in our study cohort. These findings indicate that alcohol consumption cannot be considered a risk factor for unexplained female infertility in the Sudanese population.

Interestingly, despite the established significant association between female infertility and physical activity level in the previous studies, whereby an active lifestyle and moderate exercise reduce the risk of infertility (explained and unexplained) and abortions and increase the pregnancy success rates among women who undergo any of the assisted reproduction technologies[20,21,54,55], the current study did not find a significant association between unexplained female infertility and the physical activity level in Sudanese women.

This might be related mainly to the similarity of lifestyle choices and occupation affiliations mentioned before and the generally inactive lifestyle among females in Sudan[56].

Dietary diversity and consumption of different food groups by the study participants

The present study examined the consumption patterns of the ten essential food groups described by the WHO and FAO for women of reproductive age and the number of daily meals based on a 7-d recall method (Table 4). This study didn't find any significant association ($P > 0.05$) between unexplained infertility and the consumption patterns for each of the ten essential food groups among the study participants.

This indicates that despite the fact that female reproductive performance is definitely influenced by incorrect food consumption patterns, which lead to disturbances in nutritional status, alterations in ovarian function, and a subsequent decrease in fertility as shown in previous studies[29,30,57-60], however, in Sudanese women (fertile and infertile), in line with previous studies done in the Sudanese population[61-65], which found a similarity in food consumption patterns among women in Sudan, there was no difference in food consumption patterns between the two study groups.

Risk factors of unexplained female infertility in Sudanese women

This study used binary logistic regression to assess the unexplained infertility predictor factors among Sudanese women. According to the findings in Table 4 and Figure 2, only the place of residence, marital status, previous miscarriage or abortion, family history of infertility, family history of unexplained infertility, use of modern contraceptives, smoking, caffeine consumption, physical activity level, meals consumed, dairy, dark green leafy vegetables, other vitamin A-rich fruits, and vegetables. However, after controlling the effects of potentially confounding variables using multivariable logistic regression analysis, only marital status, family history of infertility, use of modern contraceptives, smoking, caffeine consumption, physical activity level, meals consumed, other vitamin-A-rich fruits and vegetables, and other vegetables were found to be significant factors associated with unexplained infertility among Sudanese women.

In the present study, married women were 0.073 times more likely to have unexplained infertility in comparison to divorced and widowed women. This can be explained by the fact that most Sudanese women seeking infertility services are married women (99.1%), so it makes sense that the odds of unexplained infertility are greater among them.

Also, women with a family history of infertility were 3.257 times more likely to have unexplained female infertility compared with other women who didn't have any family history of infertility. Similar findings were reported in studies from Iran and France, which found that women with a history of infertility in their family were 3.88 times more likely to develop unexplained infertility compared with women who didn't have any of their close relatives who had infertility before[21,23].

In light of this finding, it is essential to note that there is a strong argument that can be made about the effect of inherited "unknown genes" on female infertility. However, this argument needs more supporting evidence to make definitive conclusions.

Women who did not use any contraceptives (prior or current) had significantly higher odds of developing unexplained female infertility compared with those who used them for any period of time. Nevertheless, it can't be said with complete confidence that the use of contraceptives has contributed to the development of unexplained infertility cases in Sudanese women. Because contraceptives are used in women to prevent pregnancy from unprotected vaginal sex during fertile days, there is no need to use them in infertile women; thus, women who suffer from infertility usually do not use contraceptive methods[12,26]. This result corroborates all existing findings[13,17,23,25,35,66].

In this study, approximately 11% of Sudanese women of reproductive age smoke nicotine products, which is in line with the overall prevalence of smoking in the Sudanese population.

When compared to non-smokers, smoking women had 1.276 times the risk of developing unexplained female infertility.

This indicates that smoking has adverse effects on the normal female reproductive process, whereby the evidence specifically suggests that the smoking process produces some toxins that affect the folliculogenesis and steroidogenesis in the ovary and the FSH and LH secretion from the pituitary gland[67]. As a result, women are advised not to smoke to increase their fertility and their ability to get pregnant.

This result is consistent with a recent committee opinion report of the American Society for Reproductive Medicine[67], and other comprehensive studies about the negative effect of smoking on female fertility[14,18,68,69].

Women who had a sedentary lifestyle were more likely to have unexplained female infertility in comparison to participants with other lifestyles. This is consistent with recent findings that increasing physical activity level has a negative significant association with unexplained female infertility, with sedentary women 3.61 times more likely than moderately and very active women[21]. This finding may be explained by two main mechanisms. Sedentary physical activity in women may lead to an increase in leptin expression levels in the hypothalamus-pituitary-ovarian (HPO) axis, which has a negative effect on normal HPO axis function and may result in poor-quality eggs. Hence, an increase in physical activity leads to a decrease in leptin expression levels in the HPO axis and the resumption of ovulation by regulating HPO axis activity[70-73].

Additionally, high physical activity combined with the consumption of a healthy diet in women with infertility can decrease insulin and free androgen levels, leading to the restoration of the normal

function of HPO by increasing the ovarian sensitivity to gonadotropins (LH and FSH) and resulting in recovery from infertility[20,74,75].

Women who consumed more than two meals per day had a 1.606 greater probability of developing unexplained infertility compared with other women who consumed three or more meals per day. In addition, women who didn't consume vitamin-A-rich fruits and vegetables and other vegetables had higher odds of having unexplained female infertility in comparison to those who consumed these food groups in any amount.

The current finding is corroborated by the findings of the American Society for Reproductive Medicine about the relationship between diet and female fertility[76].

The three food groups mentioned—vitamin A-rich fruits, vegetables, and other vegetables—are the main source of micronutrients (vitamin A, vitamin C, vitamin E, magnesium, zinc, phosphorus, and folic acid) in Sudanese women's diet; therefore, the lack of consumption of these food groups without compensation from an external source led to a deficiency in these micronutrients. Several studies have proven that vitamins A, C, and E, magnesium, zinc, phosphorus, and folic acid play an essential role in the normal female reproductive process; any deficiency in these substances leads to disturbances in the normal female reproductive process, resulting in infertility[60,76-79].

It is important to note that insufficient nutrient intake, in general, can contribute to female infertility. This may be due to the fact that unquestionably women of reproductive age are often nutritionally vulnerable because of the dynamic way in which their bodies work and the high physiological demands, especially during the menstrual cycle. Thus, they require a more nutrient-dense diet[28].

Finally, this study showed that as caffeine consumption increases, the odds of having unexplained female infertility decrease. Studies on the effect of caffeine consumption on female fertility are inconsistent, with several studies showing negative effects of caffeine on fertility, while other studies show no association or even a significant improvement in female fertility with caffeine consumption, as summarized in a systematic review done by Bu and his colleagues in 2020[80].

The findings of the current study can be explained in light of the fact that there may be some sort of unknown negative interaction between caffeine consumption (external causes) and the female reproductive system (internal causes), which increases the risk of infertility. Although this conclusion is not conclusive, we recommend that any woman who desires to have a baby consume caffeine wisely.

To recapitulate all the above, married women with a family history of infertility who smoke and consume a high amount of caffeine, who have a sedentary lifestyle, and who consume more than two meals free of vitamin-A-rich fruits and/or vegetables and/or other vegetables per day are at the highest risk of developing unexplained infertility. Therefore, any woman who intends to get pregnant and has one or more of these risk factors needs to get an immediate consultation from a certified fertility doctor to find out early if she has unexplained infertility or not and to know the best strategies to deal with it. Also, those women need to change their diet and lifestyle to decrease the likelihood of developing unexplained infertility.

Strengths and limitations of the study

This matched case-control study provided a useful characterization of unexplained female infertility in Sudan; this, along with the control of confounding factors and low loss to follow-up, contributes to the current study's strength.

Additionally, the study outcomes and arguments were based on relatively large sample size. Hence, its conclusion could be generalizable to other settings. We were constrained by the objectives of the significant study from which this study emanated. As such, some clinical details of the women with unexplained infertility were not captured. The data used for this study were obtained through patient-reported interviews, and some responses appear retrospective; thus, the participants' responses could not be validated. However, efforts were made as much as possible to validate the information reported by participants through the patient records, especially for the participants who were not first-time visitors to the study recruitment clinics.

CONCLUSION

In conclusion, the median duration of unexplained infertility in this study was 3 years, with a range of 1–15 years. Furthermore, married women with a family history of infertility who smoke and consume a high amount of caffeine, who have a sedentary lifestyle, and who consume more than two meals free of vitamin-A-rich fruits and/or vegetables and/or other vegetables per day are at the highest risk of developing unexplained infertility.

ARTICLE HIGHLIGHTS

Research background

Unexplained infertility remains one of the mysteries in the reproductive health field, where the diagnostic evidence is still weak and the proposed treatments still work with unknown methods.

Research motivation

Unexplained infertility remains one of the mysteries in the reproductive health field, where the diagnostic evidence is still weak and the proposed treatments still work with unknown methods.

Research objectives

The objective of this study was to characterize and identify factors associated with unexplained infertility in Sudanese women.

Research methods

A matched (age and body mass index) case-control study was conducted from March 2021 to February 2022. The study samples were 210 women with unexplained infertility and 190 fertile women of reproductive age who were attending the maternity hospitals and fertility clinics in Khartoum, Sudan. The risk factors for unexplained infertility were identified using a structured, pre-tested questionnaire.

Research results

Infertile women had a significantly higher proportion of family history of infertility (explained and unexplained) compared with controls. Also, only marital status, family history of infertility, use of modern contraceptives, smoking, caffeine consumption, physical activity level, meals consumed, other vitamin-A-rich fruits and vegetables, and other vegetables were found to be significant ($P < 0.05$) factors associated with unexplained infertility among Sudanese women.

Research conclusions

Married women with a family history of infertility who smoke and consume a high amount of caffeine, who live a sedentary lifestyle, and who consume more than two meals free of vitamin-A-rich fruits and/or vegetables and/or other vegetables per day are at the highest risk of developing unexplained infertility.

Research perspectives

More interventional studies regarding the main factors, such as physical activity and dietary intake, for infertile women with unexplained causes, need to be done.

FOOTNOTES

Author contributions: Abdullah AA, Ahmed M, and Oladokun A analyzed and interpreted the data, and drafted the manuscript; Abdullah AA and AO designed the study and directed implementation and data collection; Abdullah AA, Ahmed M, and Oladokun A edited the manuscript for intellectual content and provided critical comments on the manuscript; All authors gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

Institutional review board statement: The study protocol and procedures were approved by the University of Ibadan/University College Hospital (UI/UCH) Ethics Committee (Ref. No; UI/EC/20/0438), and the Federal Ministry of Health, Sudan republic (Ref. No; 4-12-20).

Informed consent statement: Informed consent was obtained from all the study participants, and all necessary information regarding the study (objectives, requirements of the participants, and duration of the study) was given to the prospective study participants on an information sheet in Arabic to ensure an informed decision to participate in the study. Then, the full case histories of the participants were obtained through clinical examinations and laboratory investigations. Ethical principles such as discretion and confidentiality, the interviewees' free consent, and beneficence and nonmaleficence to participants were strictly adhered to.

Conflict-of-interest statement: The authors declared no potential conflict of interest concerning the research, authorship, and/or publication of this article.

Data sharing statement: The datasets used during this study are available from the principal investigator upon reasonable request.

STROBE statement: The authors have read the STROBE Statement – checklist of items, and the manuscript was

prepared and revised according to the STROBE Statement – checklist of items.

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Country/Territory of origin: Sudan

ORCID number: Abdullah Abdulslam Abdullah 0000-0003-2534-4953; Musa Ahmed 0000-0003-0224-9718; Adesina Oladokun 0000-0002-5740-7608.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Epidemiological trends in acute pancreatitis: A retrospective cohort in a tertiary center over a seven year period

Andreea Irina Ghiță, Mihai Radu Pahomeanu, Lucian Negreanu

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Andreea Irina Ghiță, Faculty of Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest 050474, Romania

Mihai Radu Pahomeanu, Lucian Negreanu, Internal Medicine I & Gastroenterology - University Emergency Hospital of Bucharest, Carol Davila University of Medicine and Pharmacy, Bucharest 050098, Romania

Corresponding author: Mihai Radu Pahomeanu, Doctor, Assistant Professor, Internal Medicine I & Gastroenterology - University Emergency Hospital of Bucharest, Carol Davila University of Medicine and Pharmacy, Splaiul Independenței 169, Sector 5, Bucharest 050098, Romania. mihai.pahomeanu@drd.umfcd.ro

Abstract

BACKGROUND

Acute pancreatitis (AP) remains a major cause of hospitalization and mortality with important health-related costs worldwide. Using an electronic database of a large tertiary center, we estimated the incidence, etiology, severity and costs of hospitalized AP cases in southern Romania.

AIM

To estimate the incidence, cost and tobacco usage of hospitalized AP cases in southern Romania and to update and upgrade the knowledge we have on the etiology, severity (in regard to Revised Atlanta Classification), outcome, morphology and local complications of AP.

METHODS

We performed an electronic health care records search on AP patients treated at Emergency University Hospital of Bucharest (Spitalul Universitar de Urgență București) between 2015 and 2022. The incidence, etiology, and severity were calculated; potential risk factors were evaluated, and the hospitalization costs of AP were documented and analyzed. The cohort of this study is part of the BUCharest - Acute Pancreatitis Index registry.

RESULTS

A total of 947 consecutive episodes of AP where the patients were hospitalized in the gastroenterology department were analyzed, with 79.45% as 1st episode and the rest recurrent. The majority of the patients were males (68.9%). Alcoholic (45.7%), idiopathic (16.4%) and biliary (15.2%) were the main causes. The

incidence was estimated at 29.2 episodes/100000 people. The median length of stay was 7 d. The median daily cost was 747.96 RON (165 EUR). There was a high prevalence of active tobacco smokers (68.5%). The prevalence of severe disease was 11.1%. The admission rate to the intensive care unit was 4.6%, with a mortality rate of 38.6%. The overall mortality was 5.5%.

CONCLUSION
We estimated the incidence of AP at 29.2 episodes that required hospitalization per 100000 people. The majority of our cases were found in males (68.9%) and were related to alcohol abuse (45.7%). Out of the cases we were able to find data regarding tobacco usage, the majority were active smokers (68.5%). Most patients had a mild course (54.4%), with a mortality rate of 5.5%. Interstitial AP prevailed (45.3%). The median daily cost of hospitalization was 747.96 RON (165 EUR).

Key Words: Acute pancreatitis; Epidemiology; Revised Atlanta Classification; Mortality; Outcome; Cost

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Core Tip: Retrospective cohort study on 947 consecutive hospitalized episodes (in 829 patients) of acute pancreatitis (AP) with the aim of estimating the incidence, cost and tobacco usage in AP cases and to update & upgrade the knowledge we have on the etiology, severity (as stated in Revised Atlanta Classification), outcome, morphology and complications of AP. Out of our study resulted that: The majority of patients were males (68.9%); alcoholic etiology prevailed (45.7%); estimated incidence: 29.2 episodes/100000 people; median daily cost: 165 EUR; median hospitalization 7 d; majority active tobacco smokers (68.5%); 11,1% severe disease; admission to intensive care unit 4.6%; overall mortality 5.5%.

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INTRODUCTION

Acute pancreatitis (AP) remains one of the main conditions treated in gastroenterological departments worldwide and is a common cause of hospitalization. With an incidence of 3.8-74.8 cases per 100000 in Europe[1]. AP remains a burden for health care system expenditures, although approximately 75%-80% of patients will develop only a mild clinical course[2]. Positive diagnosis is based on at least 2 positive criteria from the following: Lypasemia (or amylasemia) higher than 3 times the normal range, a clinical presentation (upper abdominal pain that radiates to the back, nausea, vomiting, *etc*.) or imaging criteria (ultrasound, contrast-enhanced computed-tomography, contrast-enhanced magnetic resonance imaging)[3].

Updated population-based studies on AP in Romania are lacking. Our aim was to evaluate the current data for AP in a tertiary center in Bucharest. The objective of this paper is to estimate the incidence, cost and tobacco usage of hospitalized AP cases in southern Romania and to update and upgrade the knowledge we have on the etiology, severity (in regard to Revised Atlanta Classification), outcome, morphology and local complications of AP. Although most of those issues have already been addressed in other populations, there is a high degree of heterogeneity in the worldwide and time-related AP demographics, etiologies, management practices, and outcomes[4]. A previous study conducted in 2005 on a similar population[5] of patients from a gastroenterological services found a dominance of alcohol-related pancreatitis. However, that study had a relatively small number of patients and could not estimate the incidence and stratify etiologies other than alcoholic, biliary, post-endoscopic retrograde cholangiopancreatography and hypertriglyceridemia.

MATERIALS AND METHODS

Patients

For this observational retrospective study, we inquired about the Spitalul Universitar de Urgență București (Emergency University Hospital of Bucharest) digital database for cases of AP [International Classification of Diseases 10 code: K85, B25.2, B26.3], in which adult patients were admitted to gastroenterological wards between 1 June, 2015 and 1 April, 2022.

The inquiry obtained 1074 consecutive episodes (Table 1). All of the patients were screened by the authors for miscoding, revealing that all 1074 fulfilled at least 2 criteria for AP (as they are mentioned in the Revised Atlanta Classification)[6], out of which 126 were chronic pancreatitis, so we excluded them from this study. We collected the following data: Sex, month of admission, age, number of days of admission, number of days of admission to the intensive care unit (ICU) (if it were the case), outcome at discharge, type of severity according to the Revised Atlanta Classification[6], type of morphology according to the Revised Atlanta Classification, probable etiology, urban-rural residence, county of origin, previous history of pancreatitis, smoking habits and cost of admittance.

Morphology was assessed according to the Revised Atlanta Classification by the authors' consensus from the available imagistic investigation. We took into consideration (arranged by the power of evidence) abdominal ultrasound, endoscopic ultrasound, contrast-enhanced computer tomography, and contrast-enhanced magnetic resonance.

This cohort is represented only by the patients admitted to the gastroenterology department and represents the BUCharest - Acute Pancreatitis Index (BUC-API) 1 - Gastroenterology cohort. For the aforementioned cohort, we took into consideration demographic, clinical, biological and imagistic data obtained from the electronic database of Emergency University Hospital of Bucharest. The population of this cohort is represented by 918 patients, with 1074 episodes of AP, recurrent AP and acute-on-chronic pancreatitis involving patients who were admitted to our department from 1 June, 2015 to 1 April, 2022 with AP. Details regarding the number of unique patients are from the BUC-API 1 - Gastroenterology cohort can be found in Table 1.

Statistical analysis

The database was organized using Microsoft Excel 2019©. For the statistical analysis of the data, we used crosstab analysis, frequency analysis, linear regression, ANOVA, χ^2 test, Fisher exact test, and goodness of fit run on the statistical program IBM SPSS Statistics version 29.0.0.0©.

RESULTS

Estimated incidence of AP

Our hospital serves as a tertiary referral center for a population of approximately 950 thousand inhabitants, and we are admitting half of the AP patients in our hospital as the other half being admitted to surgical wards. Our search identified 1074 episodes, of which 126 were miscoded as AP, being in fact acute-on-chronic pancreatitis. The remaining 947 consecutive episodes were AP to which the patients were admitted to the gastroenterological wards of our hospital in the timespan of 6 years and 10 mo, between 1st of June 2015 and 1st of April 2022. Based on the aforementioned statistics, we managed to estimate an incidence of AP in southern Romania of 29.2 episodes per 100000 people. This incidence means that we estimate approximately 5900 hospitalizations for AP annually at the country level.

Demographics

We found a total of 947 consecutive episodes that fulfilled at least 2 out of the 3 diagnostic criteria and were not chronic pancreatitis. Of them, 75.39% (n = 714) of the patients did not have any history of AP, and the others had at least one previous episode of AP but without signs of chronic disease and/or pancreatic malignancy.

In total, 68.88% (n = 652) of the cases were in male patients, and the median age was 54 years (± 15.9). By type of residence, 73.1% (n = 692) of the patients were from cities, 25.4% (n = 241) were from the countryside, and the remaining 1.4% (n = 14) did not have a fixed residence within Romania.

Etiology

We have defined the etiology of AP in regard to 16 possible causes and another 18 possible intricate etiologies, based on how they were defined as predisposing conditions in Sleisenger and Fordtrans - Gastrointestinal and Liver diseases - 10th edition[7]. We have defined some of the etiologies as follows: (1) Alcohol-related[8]: Regular alcoholic consumption (obtained through anamnesis) and/or indirect elements in cases without an apparent etiology, such as macrocytosis, icterical cholestasis, DeRitis ratio [9] > 2 in middle-aged men, Dupuytren contracture, *etc.* We could not quantify the usage of CAGE Questionnaire[10] from the medical records we reviewed; (2) Biliary: Imagistic findings (ultrasonographic, computer tomography or magnetic-resonance) with elevated aminotransferases (alanine aminotransferase or aspartate aminotransferase)[8]; (3) Hypertriglyceridemia: Triglycerides > 750 mg/dL, we sought to use a threshold formed from an average between 1000 mg/dL[8], and the one recommended for treatment of hypertriglyceridemia by ATP III guideline (500 mg/dL)[11]; (4) Trauma: Anamnesis, a high creatine kinase; and (5) Diabetes mellitus: No apparent etiology and at least one of the following: Hemoglobin A1c > 7.5% or glycemia > 250 mg/dL at two consecutive findings (without prior history of diabetes mellitus).

Table 1 Number of unique patients in the BUCharest – Acute Pancreatitis Index 1 – gastroenterology cohort

Type of disease	Number of episodes	Number of unique patients
AP & RAP	947	829
Acute-on-chronic pancreatitis	126	89
Total BUC-API 1 gastroenterology	1074	918

BUC-API: BUCharest – Acute Pancreatitis Index; AP: Acute pancreatitis; RAP: Recurrent acute pancreatitis.

All other single etiologies were classified by the authors' consensus. We found 45.7% (n = 433) of the cases to be related to alcohol consumption and 15.2% (n = 144) were related to gallstones. Among other remarkable etiologies, we found 16.4% (n = 155) idiopathic, 3.5% (n = 33) hypertriglyceridemia-related, 3% (n = 28) diabetes mellitus-related, and 2.5% (n = 25) pharmacological (Table 2).

Tobacco usage

We were able to identify tobacco usage in 40.5% of the patients (n = 384), out of which 68.5% (n = 263) were active smokers and another 22.4% (n = 86) ceased smoking tobacco more than 4 wk prior to hospitalization. We could not objectively quantify the number of pack-years from the medical records.

Severity and outcome

At discharge, we found that 54.4% (n = 515) had mild AP, 34.5% (n = 327) had a moderately severe course of disease, and the latter 11.1% (n = 105) had severe disease. A total of 4.6% (n = 44) were admitted to the ICU, with a mortality rate of 38.6% (n = 17) and a median length of stay within the ICU of 4 d (± 0.8). Regarding the entire population, the mortality rate observed was 5.5% (n = 52), with a healing rate of 83.2% (n = 788). The outcome is presented in detail in Table 3.

Regarding morphology, we retrieved information from the medical records in 73.4% of the patients. The most frequently encountered morphology was 45.3% (n = 429) who had interstitial edema, followed by 11.3% (n = 107) with a normal pancreas and 7.4% (n = 70) with acute peripancreatic collections. Necrosis as understood by acute necrotic collection and walled-off necrosis was encountered in 3.9% (n = 37) of the patients. Table 4 shows the available details about the morphology.

Hospitalization and estimated costs

The length of hospitalization varied greatly, with a median of 7 d (± 6.05) and a maximum of 101 d. Regarding the month of hospitalization, most of the patients were hospitalized in May (11.4%, n = 108), and the fewest were in hospitalized in February (n = 62). All the cases by month of hospitalization are shown in Figure 1. The median total cost was 5177.5 RON (± 6238.89) (approximately 1100 EUR), with a maximum of 100762 RON (approximately 22400 EUR). The median daily cost, was calculated to be 747.96 RON (± 411) (approximately 165 EUR). Considering the data, we were able to calculate the cost of hospitalization of the entire population included in this study at 4958226.84 RON (approximately 1 million EUR) and to estimate the annual cost of hospitalization for this disease in Romania at 30890748 RON (approximately 6.3 million EUR).

DISCUSSION

Previous reports estimated that the incidence of AP varied across Europe between 4.6 and 100 cases/100000 people annually[1,2,5,12]. We have estimated an incidence of 29.2 cases/100000 people, or approximately 5900 episodes annually throughout the entire country, which is an expected and moderate profile of incidence. We could not find any specific data about incidence in our country, so this is most likely the first attempt to estimate the incidence of AP in Romania.

To the best of our knowledge, this is the first attempt to estimate the cost of hospitalization in Romania. We observed a median total cost of 5177.5 RON (approximately 1100 EUR) and a median daily cost of 747.96 RON (approximately 165 EUR). Comparing it to other studies[12,13], we found a median daily cost similar to that in Spain (143 EUR) but far lower than the median total cost of 10069 USD in the United States in 2010. A possible limitation resides in the fact that all our patients were hospitalized in public-owned facilities, so it is possible that some of the costs were underestimated.

Smoking might be an independent risk factor for AP severity and evolution[14-16]. In our study, we were able to find that more than two-thirds of the patients smoked actively, while another 22.4% were former smokers. These data show us a higher percentage of active smokers in the AP population than those reported in the general population of Romania (68.5% *vs* 30%, as stated by the 2021 Eurobarometer). We will soon try to observe if there is any correlation between smoking tobacco products and

Table 2 Frequency of etiologies

Cause	Number of cases	Percent (%)
Alcohol	433	45.7
Idiopathic	155	16.4
Biliary	144	15.2
Hypertriglyceridemia	33	3.5
Diabetes mellitus	28	3.0
Pharmacological	25	2.6
Mixed (alcohol & biliary)	21	2.2
Mixed (alcohol & diabetes mellitus)	21	2.2
Mixed (alcohol & hypertriglyceridemia)	18	1.9
Mixed (hypertriglyceridemia & diabetes mellitus)	17	1.8
Ischemic	14	1.5
Extra pancreatic anomalies	13	1.4
Other (trauma, IBD, intrapancreatic anomalies *etc.*)	25	2.6

IBD: Inflammatory bowel disease.

Table 3 Outcome at discharge

Outcome	Number of cases	Percent (%)
Healed	788	83.2
Discharge at will	78	8.2
Deceased	52	5.5
Transferred	26	2.7
Stationary	3	0.3

Table 4 Morphology

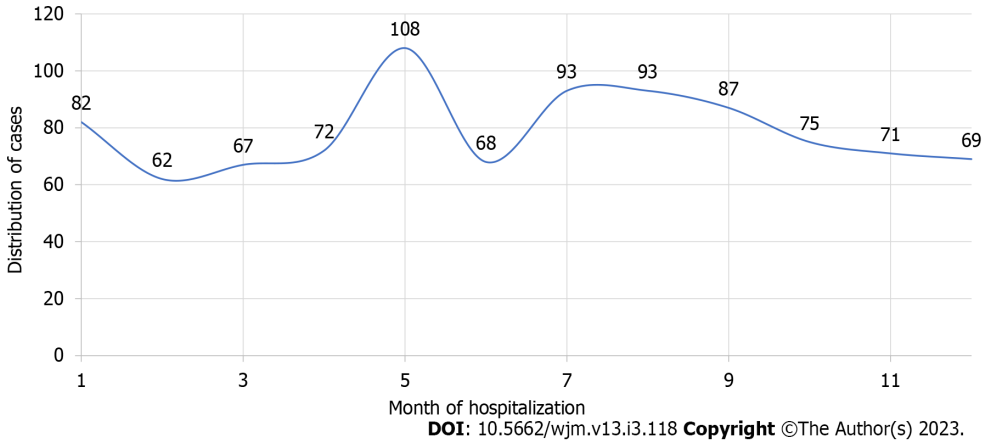
Morphology	Number of cases	Percent (%)
Interstitial	429	45.3
Normal pancreas	107	11.3
Acute peripancreatic fluid collection	70	7.4
Pseudocyst	52	5.5
Acute necrotic collection	33	3.5
Walled off necrosis	4	0.4
N/A	252	26.6

N/A: Not applicable.

AP in another paper.

The median length of stay in AP varies in the literature from 4 d in Finland[17] to 9 d in Chile[18] and 10 d in Spain[12]. We found a length of stay of 7 d, which is similar to other previously published studies. Regarding the seasonality of AP, we found a peak in incidence in May, which is somewhat similar to the findings from a Chinese study and might be related to cultural habits[19].

Previous papers found a high prevalence of alcohol-related AP with a gallstone-to-alcohol ratio of 0.39 in 2005 in Romania[2,5], similar in trend to other Eastern and Northern European countries[17,20].



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Figure 1 Distribution of cases by month of hospitalization.

In India, the gallstone-to-alcohol ratio seems to be very close to 1 (0.95), with other etiologies being negligible[21]. In Spain and the Americas[12,18,22], the gallstone etiology seems to prevail, while in China[23], although the gallstone cause prevails, hypertriglyceridemia appears to be a highly important cause (probably due to lower rates of alcoholism in that region). Globally, it seems that gallstone-related AP is the most common[24]. In our cohort of patients, alcoholic etiology was most prevalent (45.7%), with a lower than previously reported gallstone-to-alcohol ratio of 0.33 and an overall relative lower rate of all the main four etiologies (alcohol, biliary, idiopathic and hypertriglyceridemia).

In regard to the outcomes, we observed lower rates of mild AP than those of other studies that have stratified severity in regard to the Revised Atlanta Classification[18,25]. We observed a lower (4 *vs* 8 d) median length of stay in the ICU than those observed in China[26] or Australia[27] but higher mortality rates than those of China[28], Finland[17] or Germany[29]. Nevertheless, similar mortality and severity rates were observed in populations that are geographically, culturally, culinary, and genetically similar to the Portuguese[30].

CONCLUSION

We estimated the incidence of AP at 29.2 episodes that required hospitalization per 100000 people. The majority of our cases were found in males (68.9%) and were related to alcohol abuse (45.7%). Out of the patients we were able to find data regarding tobacco usage, a vast majority of the patients were active smokers (68.5%). Most of our patients had a mild course (54.4%), and the total mortality rate was 5.5%. Interstitial AP prevailed in our cohort (45.3%). 747.96 RON (approximately 165 EUR) was the median daily cost of hospitalization. This study's main strengths are based on the fact that is a large cohort study with over 1000 episodes and with a low bias risk of population selection regarding the fact that the episodes taken into account were consecutive AP cases of our department. The weaknesses of this study resides in the fact that is a retrospective, unicentric study that is based on medical-chart reviews that is prone to data loss between discharge and study analysis and also the lack of surgical patients. There is a need to extend this study to patients admitted in surgical departments to correctly evaluate prognosis and severity.

ARTICLE HIGHLIGHTS

Research background

Acute pancreatitis (AP) is a global burden, especially in Eastern Europe and former Soviet space. Romania although is part of Eastern Europe lacks quality epidemiological studies regarding the topics we aim in this study, like: Estimation of incidence, stratification of: Etiology, severity, outcome, morphology, estimation of cost regarding hospitalization of AP and tobacco usage prevalence in our country regarding AP cases.

Research motivation

From this study we aim to estimate the incidence, cost and tobacco usage in hospitalized AP cases and to upgrade and update former knowledge regarding: Etiology, severity, outcome, morphology of AP. Once this aim is fulfilled the data from this paper should be of use for: Medical practitioners from our

country and countries that have large Romanian diaspora, medical researchers and healthcare policy-makers from our country or any other international organization with a focus on this topic.

Research objectives

Main objective: Estimating the incidence was fulfilled although we were able to do that only in regard to southern Romania. Secondary objectives achieved: First estimation of AP costs in our country, first attempt to find the prevalence of tobacco usage in AP in our country. Also, we were able to update the knowledge regarding stratification. This study should be of use for a nationwide metanalysis of smaller regional studies or for a European or international metanalysis regarding this topic. This study should be also expanded with surgical patients.

Research methods

Cases drawn from BUCharest - Acute Pancreatitis Index cohort of AP and Acute-on-Chronic Pancreatitis cases, which to the best of our knowledge is the largest analysed in Romania to this date. The entire team that worked on this study, did that remotely due to coronavirus disease 2019 pandemic restriction with the help of several online applications like: Adobe Reader, Microsoft 365 (formerly known as Microsoft Excel), Google Teams, Zoom, SPSS statistical package. We analysed data from Electronic Health Records of our facility, based on International Classification of Diseases (ICD)-10 coding of diagnostics. For the statistical analysis we used IBM SPSS v. 29.0.0.0 and we run the following tests depending on the type of variable of interest: Crosstab analysis, frequency analysis, χ^2 test.

Research results

We managed to show that AP incidence total annual cost in Romania might be overestimated as stated in Global Burden of Disease study 2019 (no other recent data publicly available), this should be of great interest for healthcare policy makers in our country or other international organization interested by this topic. In the selection of cases, we also observed that ICD-10 does not have a particular code for Acute-on-Chronic Pancreatitis, this was a limitation that was dealt trough authors screening and exclusion but we consider that a code for this particular situation would be of great necessity.

Research conclusions

Our study proposes a first, as far we know, estimation regarding costs of AP in our country and also a first glance, to the best of our knowledge, regarding tobacco usage prevalence in this disease. We also managed to make a reasonable first, as far as we know, grassroots estimation of incidence of AP in our region based on the data we have at this moment.

Research perspectives

We seek in a near future to expand this study also on surgical cases and territorially as a multicentric study to be able to better estimate the current status of AP outside southern Romania.

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FOOTNOTES

Author contributions: Ghiță AI and Pahomeanu MR contributed to the collection of data, input of data, primary statistical analysis and drafting of the article; Negreanu L contributed to the article writing and final corrections; and all authors approved the final version of the article.

Institutional review board statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of Emergency University Hospital of Bucharest (Spitalul Universitar de Urgență București).

Informed consent statement: Participants gave general informed consent at admittance for medical data to be used in medical research and consent was not obtained in particular for this study but the presented data are anonymized and risk of identification is low.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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mihai.pahomeanu@drd.umfcd.ro.

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Country/Territory of origin: Romania

ORCID number: Andreea Irina Ghiță 0009-0002-8753-9924; Mihai Radu Pahomeanu 0000-0002-7052-4294; Lucian Negreanu 0000-0003-3042-0754.

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SYSTEMATIC REVIEWS

Acceptability and strategies for enhancing uptake of human immunodeficiency virus self-testing in Nigeria

Victor Abiola Adepoju, Chidinma Umebido, Ademola Adelekan, Ali Johnson Onoja

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Victor Abiola Adepoju, Chidinma Umebido, Department of HIV and Infectious Diseases, Self testing in Africa (STAR) Project, Jhpiego Nigeria, Abuja 900901, Federal Capital Territory, Nigeria

Ademola Adelekan, Department of Public Health and Biological Sciences, Blue Gate Research Institute, Ibadan 200116, Oyo State, Nigeria

Ali Johnson Onoja, Department of Research, African Health Project, Abuja 900901, Federal Capital Territory, Nigeria

Corresponding author: Ademola Adelekan, DrPH, Researcher, Department of Public Health and Biological Sciences, Blue Gate Research Institute, Ibadan 200116, Oyo State, Nigeria. ademolaadelekan@gmail.com

Abstract

BACKGROUND

In 2019, the Nigerian Ministry of Health published the first operational guidelines for human immunodeficiency virus self-testing (HIVST) to improve access to human immunodeficiency virus (HIV) testing services among undertested populations in the country. Also, as part of the campaign to increase HIV testing services in Nigeria, the Nigerian Ministry of Health developed standard operating procedures for using HIVST kits.

AIM

To systematically review the acceptability and strategies for enhancing the uptake of HIVST in Nigeria.

METHODS

The systematic review was conducted and reported in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses. Different databases were searched to get the necessary materials needed for this review. Standardized forms developed by the authors were used for data extraction to minimize the risk of bias and ensure that the articles used for the study were properly screened. Identified articles were first screened using the titles and their abstracts. The full papers were screened, and the similarities of the documents were determined. Qualitative, quantitative, and mixed-method studies were evaluated using the Critical Appraisal Skills Programme and Critical Appraisal Framework criteria.

RESULTS

All the publications reviewed were published between 2015 and 2022, with 33.3% published in 2021. Most (77.8%) of the studies were cross-sectional, 43.3% were conducted in Lagos State, and 26.3% were conducted among young people. The study revealed a high level of acceptability of HIVST. Certain factors, such as gender, sexual activity, and previous testing experience, influence the acceptability of HIV self-testing, with some individuals more likely to opt-out. The cost of the kit was reported as the strongest factor for choosing HIVST services, and this ranged from 200 to 4000 Naira (approximately United States Dollar 0.55-11.07), with the majority willing to pay 500 Naira (approximately United States Dollar 1.38). Privately-owned, registered pharmacies, youth-friendly centres, supermarkets, and online stores were the most cited access locations for HIVST. The least influential attribute was the type of specimen needed for HIVST. Strategies addressing cost and preferred access points and diverse needs for social media promotion, local translation of product use instructions, and HIVST distribution led by key opinion leaders for key populations were found to significantly enhance HIVST uptake and linkage to care.

CONCLUSION

HIVST acceptability is generally high from an intention-to-use perspective. Targeted strategies are required to improve the acceptability of HIV self-testing, especially among males, sexually active individuals, and first-time testers. Identified and proposed uptake-enhancing strategies need to be investigated in controlled settings and among different populations and distribution models in Nigeria.

Key Words: Acceptability; HIV self-testing; Uptake; Intention-to-use; Regulation; Linkage to care

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Core Tip: This is the first systematic literature review on the acceptability and uptake of human immunodeficiency virus self-testing (HIVST) in Nigeria. The findings suggested that the acceptability of HIVST is high in Nigeria. However, the actual use of HIVST in programmatic implementation was lower than expected. The use of key opinion leaders among key populations successfully increased the acceptability and uptake of HIVST. However, cost was a major barrier to the acceptability of HIVST. More studies are required to evaluate how the uptake of HIVST compares in routine programs *vs* real-life settings in the absence of support and resources that enhance HIVST uptake.

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INTRODUCTION

Nigeria is ranked second among the countries with a high burden of human immunodeficiency virus (HIV) in the world[1]. In 2018, the national prevalence of HIV was 1.5%, with an estimated 1.9 million people living with HIV/acquired immune deficiency syndrome (AIDS), of which only 30.0% were on antiretroviral therapy[2]. According to the Nigeria HIV/AIDS Indicator and Impact Survey, the national HIV prevalence rate in the group of 15-49 years of age was 1.4%, with a population of 201 million in 2019[3]. In Nigeria, 53000 people died of HIV/AIDS in 2018, while the rate of HIV/AIDS-related deaths appears to have remained constant in recent years, owing to the ongoing problem of advanced HIV disease[4].

Despite increased scientific and medical advancement in the understanding and management of HIV, a large number of those infected remains untested and unaware of their serostatus[5]. One of the reasons for the poor coverage of conventional health facility-based counselling and testing is the refusal to test due to the fear of societal stigma and discrimination that may result from a positive HIV test result[6] and the fear of long-term treatment, which may affect the quality of life of those infected[7].

The World Health Organization recommends HIV self-testing (HIVST) as a tool for improving the uptake of HIV testing services and achieving the Joint United Nations Programme on HIV and AIDS 90-90-90 target[8]. HIVST is an unconventional and innovative strategy to reach the first 90% goal of the United Nations Programme on HIV and AIDS by facilitating access to testing for early detection and prevention of HIV transmission[9]. Evidence shows that the deployment of HIVST has improved the uptake of HIV testing among men[10-13] in several countries implementing HIVST in Sub-Saharan

Africa including South Africa, Zimbabwe, and Botswana[10-12].

Nigeria has identified the need to increase HIV counselling and testing, including the potential of a self-testing methodology[14]. In 2019, the national AIDS and sexually transmitted diseases control programme under the Federal Ministry of Health developed the operational guidelines for the delivery of HIVST in Nigeria. The document provides guidance for the operationalization of HIVST in Nigeria including the different service delivery and distribution models, procurement and supply chain management, monitoring, and evaluation among others[5]. HIVST addresses the gap in HIV testing, especially in clinical settings. Surveys conducted among diverse populations in Malawi, Spain, the United States, and Nigeria showed varying interest in HIVST and acceptability ranges between 22% and 88%[15-19]. There is no study that systematically documented evidence either on the acceptability of HIVST or the proposed strategies to enhance its uptake in Nigeria. HIVST, as an innovative tool, is still a growing intervention in Nigeria with potential barriers to its acceptance among populations and settings. This study, therefore, aimed to systematically review the acceptability, existing regulations, and strategies for enhancing the uptake of HIVST in Nigeria.

MATERIALS AND METHODS

Design

A systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Figure 1).

Search strategy

Different databases were searched to get the necessary materials needed for this review. A scientific literature search was performed using Elsevier, Google Scholar, EMBASE, PubMed, Ovid, and Scopus databases. Grey literature was also searched from Google and Google literature, the largest databases for grey literature. Additionally, literature was systematically searched from ResearchGate, Cochrane library, and Directory of Open Access Journal. For studies that may have been missed in the electronic search, cross reference was undertaken using reference lists of all identified articles. The first search was conducted between April 4-8, 2022, while the second took place between April 15-20, 2022. Detailed inclusion and exclusion criteria were cautiously developed to match the review questions and have sufficient details to pinpoint all relevant studies and exclude irrelevant studies[20]. The literature search combined specific keywords with Boolean operators (Table 1). Although the Reference Citation Analysis tool was available for use, it was not utilized for this review. This decision was formed based on the nature of the research question and the inclusion and exclusion criteria developed for the review, which ensured that all relevant studies were identified through the comprehensive search strategy described above.

Inclusion criteria

Both qualitative and quantitative studies on HIVST in Nigeria were included in this study.

Exclusion criteria

Articles were excluded if no data was found for the desired outcome. Editorials and short commentaries were also excluded. Papers that were not peer-reviewed and those that the full text could not be assessed were also excluded.

Data extraction

Standardized forms developed by the authors were used for data extraction to minimize the risk of bias. One of the authors extracted data from the included studies, while the other authors checked these datasets. Discrepancies were resolved by referring to the original studies. Data on acceptability, existing regulatory context, and preference level for HIVST in Nigeria were extracted. Other data extracted include the level of uptake, linkage to treatment, and strategies for enhancing the uptake of HIVST in Nigeria. Adelekan A and Adepoju VA independently evaluated the potential eligibility of each of the abstracts and titles from the retrieved citations after requesting full-text versions of these potentially eligible studies. Onoja AJ and Umebido C independently assessed the full text of the potentially eligible publications. Disagreements were resolved by consensus. Discrepancies were discussed between authors until a 100.0% agreement was achieved. The following information was extracted from the included studies: Authors, title, study population, study state, study objective(s), study design, and study findings.

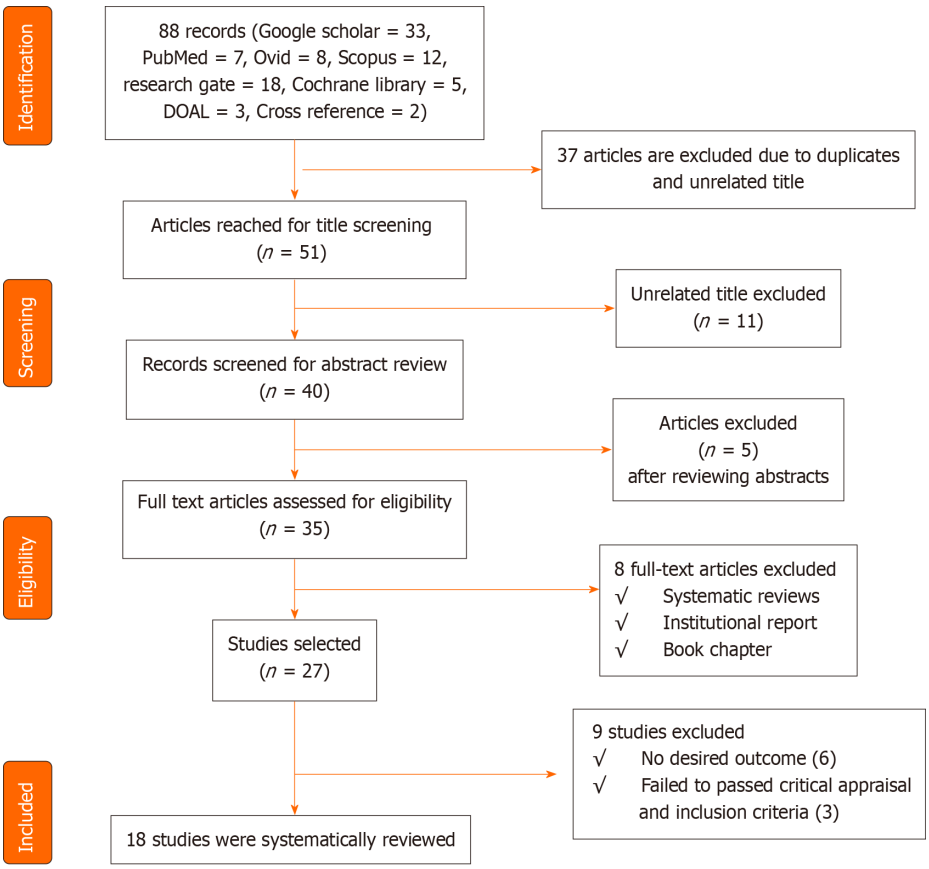
Quality assessment

The articles used for the review were properly screened. They were first screened using the titles and their abstracts. The full papers were screened, and the similarities of the papers were determined by reading the title, author(s), and abstract. The papers were then de-duplicated. The quality assessment of

Table 1 Search terms used in the literature search on human immunodeficiency virus self-testing uptake in Nigeria

Search terms	And	And
HIV self-testing	Acceptability	Nigeria
HIV regulatory	Nigeria	Self-testing
HIV self-testing	Preference	Nigeria
Nigeria HIV uptake	Self-testing Nigeria	
HIV self-testing	Nigeria treatment	Linkage

HIV: Human immunodeficiency virus.



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Figure 1 Preferred reporting items for systematic reviews and meta-analyses flow chart of the study. DOAJ: Directory of Open Access Journals.

each study selected was based on set criteria[21,22].

Critical appraisal

The qualitative, quantitative, and mixed-method research were evaluated using the critical appraisal skills programme instrument[23] and critical appraisal framework criteria[24,25].

Data analysis

In compiling and summarizing the findings of the included studies, the researchers employed a variety of methodologies. Cleaning of data in the extraction sheet was an important step before analysis. The researchers structured the data from the extraction sheet into a format that analytical tools could read. The analysis was divided into qualitative and quantitative. Quantitative data analysis involved descriptive and narrative data. This technically followed the process of classification and tabulations. Content analysis technique was used for the qualitative data analysis.

List of papers reviewed

The search results are shown in Figure 1, along with a synopsis of the papers consulted (the PRISMA flow chart). Although the databases contained 88 research articles, only 18 met the inclusion criteria for this systematic review (Table 2).

RESULTS

The included studies were published between 2015 and 2022, with 33.3% published in 2021 (Figure 2). Majority (77.8%) of the studies were cross-sectional in design (Figure 3), 43.3% were carried out in Lagos State (Figure 4), and 26.3% were conducted among young people (Table 3).

Acceptability of HIVST in Nigeria

This review operationally defined the acceptability of HIVST as an intention to use, willingness to use, actual collection, and interest in use. The findings of most studies from Nigeria[5,7,26-31] revealed a high level of acceptability of HIVST compared to what was reported by a study from Northern Nigeria [15] (Figure 5). Brown *et al*[27] reported that 54.8% of the respondents supported having HIVST in Nigeria. Adebimpe *et al*[7] also reported that 86.0% of the respondents agreed that they would accept HIVST if kits were available, and 84.0% agreed that they would be willing to introduce and recommend HIVST to others. In another qualitative study by Dirisu *et al*[28] it was observed that most participants were willing to use oral HIVST kits.

Iliyasu *et al*[15] found that 70.4% of university students in northern Nigeria were willing to self-test for HIV and pay for the test kits. Specifically, 55.9% of the participants were willing to pay for the test kits themselves, and 14.5% were willing to pay for the test kits if they were cheaper. Additionally, 61.4% of the participants were willing to self-test with a sexual partner[15]. Also, Ugwu *et al*[29] reported that 61.3% of the health workers working in primary health care centres preferred HIVST over the facility-based testing modalities. In 2021, a study also reported that 59.3% of the respondents were interested in HIVST[30]. In another study, it was observed that nearly all (99.5%) of the pregnant women enrolled in the study preferred conventional HIV testing service at booking. However, 83.8% of these pregnant women were keen to learn how to self-test for HIV, and 85.7% of the respondents were willing to repeat the HIV test during pregnancy, of which 29.3% were willing to self-test[31]. Similarly, 94.6% of respondents were willing to retest for HIV after delivery, of which 27.4% were willing to self-test.

However, another study among young people in Nigeria noted that more than half (69.9%) of the participants indicated they would prefer a physician to administer the HIV test. In comparison, the proportions of those who preferred HIV tests administered by a nurse and self-administered HIV tests were 15.7% and 4.8%, respectively. Another study from Southern Nigeria reported that 23.4% of the respondents accepted HIVST, of which 33.3% of the clients were assisted[5]. In terms of preference of oral *vs* blood-based HIVST in Nigeria, another study by Obiezu-Umeh *et al*[32] reported that oral-based HIVST was preferred by most of the young participants when compared to blood-based HIVST.

Ong *et al*[33] used a discrete choice experiment to design HIVST services for young people in Nigeria. The authors reported that male individuals (compared with female individuals), those who never had sex (compared with sexually active), and those who had never tested for HIV before (compared with those who had previously tested) were more likely to opt-out of using an HIVST kit.

Existing regulatory context for HIVST

Only one study examined the regulatory context for HIVST in Nigeria. The study by Dirisu *et al*[34] examined the regulatory framework for HIVST in Nigeria, revealing several issues. Of the providers who marketed HIVST kits, 94.0% of community pharmacists (CPs) and 33.0% of patent proprietary medicine vendors (PPMVs) claimed to be authorized to sell them[34]. Despite the existence of a National Drug Policy and an automated product registration system administered by the National Agency for Food and Drug Administration and Control, the process for authorizing, manufacturing, and distributing medical products was reported as cumbersome, time-consuming, and costly[34]. Furthermore, the National Drug Distribution Guideline was not implemented, leading to an uncoordinated supply chain. The study also found that less than half (45.6%) of PPMVs and CPs had a standard operating manual for administering HIVST, and about one-third had standard guidelines for HIV testing services. While 77.0% of providers offered counselling before selling HIVST, only 23.0% of CPs and 13% of PPMVs that sold HIVST were accredited HIV counselling and testing centres[34]. These findings demonstrate the need for improved regulatory oversight and support for HIVST implementation in Nigeria.

Uptake of HIVST and linkage to care services

A cohort study on the uptake of HIVST revealed that 97.0% had used self-testing kits. Among these, almost a quarter (22.7%) tested themselves the same day they received the kit, and 49.4% tested within 1 week. About one-quarter (23.5%) reported that they had someone else present while they tested. Of

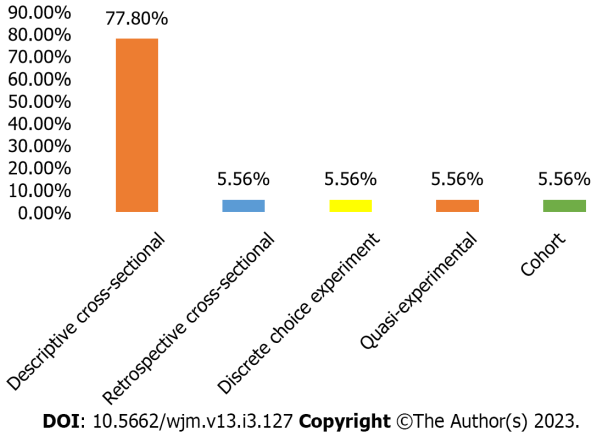
Table 2 List of papers reviewed

Ref.	Title	Study population	State of study	Objectives/research question	Study design	Findings
Adebimpe *et al*[7], 2019	How acceptable is the HIV/AIDS self-testing among women attending immunization clinics in Effurun, Southern Nigeria	All women of reproductive age (15-49 year) attending the immunization clinic (for their children) in Ekpan General Hospital	Delta	Assess the knowledge and acceptability of HIVST among women of childbearing age attending immunization clinics in Effurun, Southern Nigeria	Descriptive cross-sectional study	The study respondents' high knowledge levels and acceptability of HIVST lend support to the fact that the procedure should be promoted in the stakeholders' efforts to improve HIV testing among the general population
Adeoti *et al* [30], 2021	Sexual practices, Risk perception, and HIV Self-Testing acceptability among long-distance truck drivers in Ekiti State, Nigeria	Adult male long-distance truck drivers in Ado-Ekiti, Southwestern Nigeria	Ekiti	Examined the sexual practices, risk perception, and HIVST acceptability among long-distance truck drivers in Ekiti State, Nigeria	Cross-sectional study	Many long-distance drivers were engaged in unsafe sexual practices and were at risk for HIV transmission. Increasing testing using HIVST has the potential to bridge the gap in the diagnosis of HIV among long-distance drivers who are willing to be tested
Brown *et al* [27], 2015	HIVST in Nigeria: Public opinions and perspectives	Researchers, academics, journalists, community advocates, activists, and HIV policy-makers and programmers, including those working in the development sectors, enlisted on the new HIV vaccine and microbicide advocacy society listserv	All states	Obtained perspectives of informed members of the Nigerian public on the use of the HIVST	Cross-sectional study	Cost-based pricing can be based on and directly tied to current product experiences and information as well as how crucial product monitoring is when pricing a product
Dirisu *et al* [28], 2020	'I will welcome this one 101%, I will so embrace it': A qualitative exploration of the feasibility and acceptability of HIV self-testing among MSM in Lagos, Nigeria	MSM	Lagos	Explored MSM perceptions of oral HIVST and potential barriers to and facilitators of HIVST use. In addition, it sought to identify operational and contextual issues that might affect the distribution of HIVST kits to MSM in the Nigerian context and the potential for linkage to care	Qualitative descriptive study	The potential of HIVST to increase the uptake of HIV testing among MSM in Nigeria was supportive of HIVST. Privacy and convenience offered by HIVST address concerns about stigma and waiting times associated with facility-based testing
Iliyasu *et al* [15], 2020	Acceptability and correlates of HIV self-testing among university students in northern Nigeria	University students	Kano	Examine the acceptability of HIVST and identify factors associated with the uptake of HIVST services among university students in Kano, Nigeria	Cross-sectional study	HTS uptake was low among a sample of university students in northern Nigeria, but most university students were willing to self-test for HIV
Iwelunmor *et al*[36], 2020	The 4 youth by youth HIV self-testing crowdsourcing contest: A qualitative evaluation	All young people between the ages of 10 year to 24 year in Nigeria	All states	Describe the responses to a crowdsourcing contest aimed at soliciting ideas on promoting HIVST among young people in Nigeria	Qualitative study	The study informed the development of innovative youth implementation strategies to increase the uptake of HIVST among adolescents and youth at risk for HIV
Agada *et al* [5], 2021	Reaching out to the hard-to-reach populations with HIV self-testing services in South-south Nigeria	General population	Cross River and Akwa-Ibom	Assess the impact of the total market approach deployed in Cross River and Akwa Ibom States in South-south Nigeria to enhance the demand for HIVST to ensure product equity, accessibility, and sustainability	Retrospective cross-sectional study	The HIVST model demonstrated the potential to be a vital tool in expanding HIV testing services and linking HIV care services to populations who would otherwise not have been tested
Dirisu *et al* [34], 2020	Exploring the regulatory context for HIV self-testing and PrEP market authorisation and use in Nigeria	PPMVs and CPs	Abuja, Rivers, Imo, Lagos, and Ogun	Assess HIVST/PrEP availability and market authorization; determine the facilitators and barriers to access; and identify existing systems that support the availability, appropriate use,	Cross-sectional study	About 63% of CPs and 27% of PPMVs sold HIVST kits, while 15% of CPs and no PPMV sold PrEP in their facilities. Most CPs (94%) and 33% of PPMVs who sold HIVST kits reported that their facilities were

				affordability, and accessibility in the private sector in Nigeria		authorized to sell HIVST kits
Nwaozuru *et al*[26], 2019	Preferences for HIV testing services among young people in Nigeria	Youth aged 14–24 year	Lagos	Assessed preferences for HIV testing options among young people in Nigeria	Cross-sectional study	HIV testing services was optimized to reach young people in various options to meet their unique preferences
Ong *et al* [33], 2021	Designing HIV Testing and Self-Testing Services for young people in Nigeria: A discrete choice experiment	Nigerian youth (14-24 year)	Lagos	Examine the strength of Nigerian youth preferences related to HIV testing and HIVST	Discrete choice experiments	There could be demand for HIVST for Nigerian youth, who prefer HIVST kits that integrate testing for other STIs and is accessed from community health centres
Obiezu-Umeh *et al* [32], 2021	Young people's preferences for HIV self-testing services in Nigeria: A qualitative analysis	Young people (14–24 year)	Lagos	Use qualitative methods to examine HIVST preferences among Nigerian youth	Cross-sectional study	HIVST preferences among Nigerian youth appeared to be influenced by several factors, including lower cost, less invasive testing method, location of testing, and linkage to care and support post-testing. Findings underscored the need to address young people's HIVST preferences as a foundation for implementing programs and research to increase the uptake of HIVST
Obiezu-Umeh *et al* [51], 2020	Development of HIVST services through youth engagement: A qualitative evaluation of a health designation in Nigeria	Young people (14–24 year)	Lagos	Explore strategies for HIVST delivery developed at a designations contest in Nigeria	Cross-sectional study	Designations were a feasible method of facilitating meaningful youth engagement to develop deployable strategies to increase the uptake of HIV testing in young people in Nigeria
Durosinmi-Etti *et al* [38], 2021	Communication needs for improved uptake of PrEP and HIVST services among key populations in Nigeria: A mixed-method study	MSM, FSWs, and key influencers of the KP groups (health providers, peer educators, HIV program officers)	Akwa Ibom, Cross River, and Lagos	Identify the communication needs and preferences of the KP groups as evidence for developing strategies and interventions to increase awareness and use of HIVST and PrEP services among the KPs in Nigeria	Cross-sectional study	KPs effectively networked to increase awareness and access to PrEP and HIVST services in Nigeria. They will make the peers receptive to the interventions and help them reach other peers in their network, especially the hard-to-reach
Sekoni *et al* [37], 2022	Operationalizing the distribution of oral HIVST kits to MSM in a highly homophobic environment: the Nigerian experience	MSM and KOL	Lagos	Explore the operationalization of using KOLs to distribute HIVST kits to MSM	Cross-sectional study	This study showed the practical steps involved in operationalizing the KOL support system distribution of HIVST that positively influenced the testing experience for the participants irrespective of their HIV status and engagement in care. KOLs were a reliable resource to leverage for ensuring that HIVST kit was utilized, and HIV-positive individuals were linked to treatment and care in homophobic environments
Iwelunmor *et al*[52], 2022	Enhancing HIVST among Nigerian youth: Feasibility and Preliminary Efficacy of the 4 youth by youth study using crowdsourced youth-led strategies	Youth (14-24 year)	Lagos, Enugu, Ondo, and Oyo	Examine the feasibility and efficacy of crowdsourced youth-led strategies to enhance HIVST and STI testing	Quasi-experimental	The study provided promising evidence of efficacy that youth-led, crowdsourced strategies led to higher uptake of HIV and STI testing
Tun *et al* [35], 2018	Uptake of HIVST and linkage to treatment among MSM in Nigeria: A pilot programme using key opinion leaders to reach MSM	Males (17-59 year)	Lagos	Assess the feasibility, acceptability, uptake of HIVST, and linkage to HIV treatment among MSM through KOLs in Lagos, Nigeria	Cohort study	HIVST distribution through KOLs was feasible, and oral self-testing was highly acceptable among this urban MSM population. This study showed that linkage to treatment could be achieved with active follow-up and access to a trusted MSM-

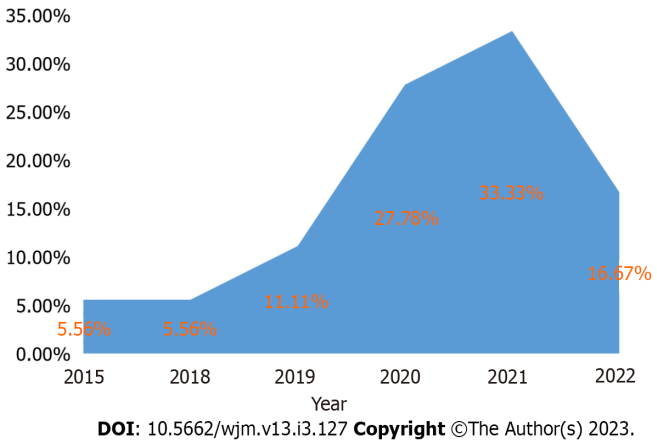
						friendly community clinic that offers HIV treatment. HIVST should be considered an additional option to standard HIV testing models for MSM
Ugwu *et al* [29], 2020	HIVST: Perspectives from primary healthcare workers in Enugu State, Southeast Nigeria	Health workers in the primary health facilities	Enugu	Assess issues surrounding the HIVST from the perspectives of the primary healthcare workers in Enugu State	Cross-sectional study	Most of the primary healthcare workers in Enugu State had poor knowledge of HIVST
Iliyasu *et al* [31], 2022	HIVST and repeat testing in pregnancy and postpartum in Northern Nigeria	Pregnant women	Kano	Determine the predictors of willingness to self-test for HIV when retesting in pregnancy and postpartum among antenatal clients at a large teaching hospital in Northern Nigeria	Cross-sectional	The acceptability of HIVST for repeat testing in pregnancy and postpartum was low, but most respondents desired to be trained to self-test for HIV

AIDS: Acquired immune deficiency syndrome; CPs: Community pharmacists; HIV: Human immunodeficiency virus; HIVST: HIV self-testing; MSM: Men who have sex with men; HTS: HIV testing services; FSWs: Female sex workers; KOL: Key opinion leader; KP: key population; PPMVs: Patent and proprietary medicine vendors; PrEP: Preexposure prophylaxis; STI: Sexually transmitted infection.



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Figure 2 Study design.



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Figure 3 Year of publication.

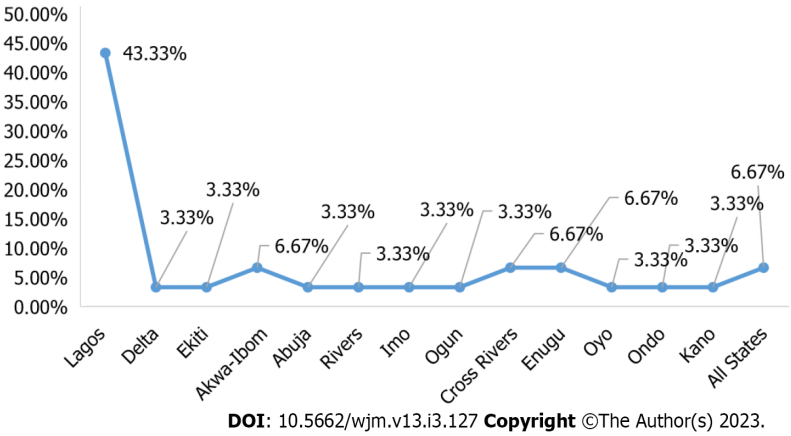
these, 55.0% tested with a friend, 21.7% tested with a family member, 16.7% tested with a sex partner, and 6.7% tested with a key opinion leader (KOL)[35]. Another study revealed that 9.0% of the respondents reported previous HIVST[15] (Figure 6).

Regarding linkage to care after HIVST, it was reported that in Cross River State 14 of the 15 clients who reported reactive results (93.3%) were linked to confirmatory testing. Of the 14 linked to confirmatory testing, 13 (91.0%) were confirmed positive, and all (100.0%) were linked to HIV treatment

Table 3 Target population for the included studies

Population	*n*	Frequency, %
Young people aged 15-24 yr	5	26.3
MSM	3	15.8
Key population influencers	2	10.5
Women of reproductive age	1	5.3
Long distance drivers	1	5.3
Professionals[1]	1	5.3
Student at university	1	5.3
General population	1	5.3
PPMV and CP	1	5.3
FSW	1	5.3
Males aged 17-59 yr	1	5.3
Pregnant women	1	5.3

[1]Ethicists, researchers, those in the academia, journalists, community advocates, activists, and policymakers.
CP: Community pharmacists; FSW: Female sex workers; MSM: Men who have sex with men; PPMV: Patent proprietary medicine vendors.



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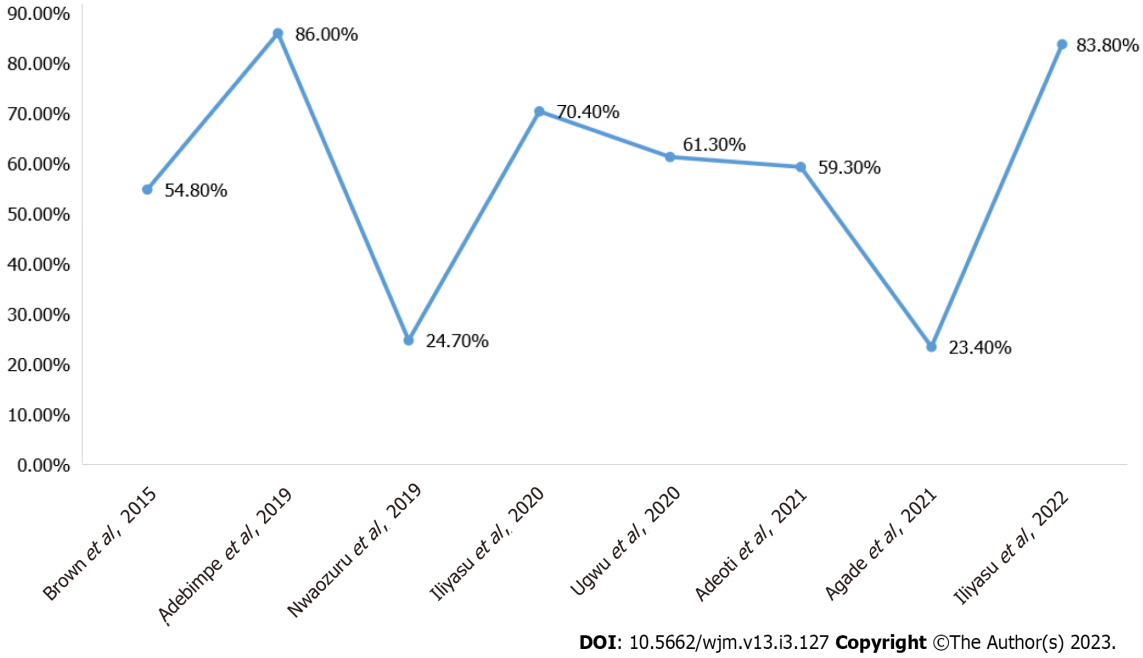
Figure 4 Study area in Nigeria.

[5]. Of the 24 who reported reactive results in Akwa Ibom State, 87.5% had confirmatory testing, 100.0% reported confirmed HIV-positive results, and 100.0% were successfully linked to HIV care and treatment[5]. Another study reported that the 14 participants who had a reactive HIV self-test sought post-test counselling and had confirmatory HIV testing at the community health centre[35].

Dirisu *et al*[34] highlighted barriers to linkage to care, including concerns around post-test counselling services and linkage to confirmatory HIV testing services following reactive HIVST results. For instance, men who have sex with men (MSM) were concerned that because self-testers would be testing alone, many would deny their HIV-positive test results and may not seek HIV treatment. In another interview that sought the opinions of the public and that of stakeholders and policy makers on the introduction of HIVST in Nigeria, participants expressed concerns about how to link individuals who tested HIVST reactive to confirmatory HIV testing services and care and treatment services as in the facility-based testing model[27]. Obiezu-Umeh *et al*[32] highlighted the motivations to seek a confirmatory HIV test in the event of a reactive HIVST result to include encouragement from peers, family members, or healthcare workers, denial about the initial HIVST test result, lack of satisfaction with the test result, and the possibility of living longer if initiated on treatment and care.

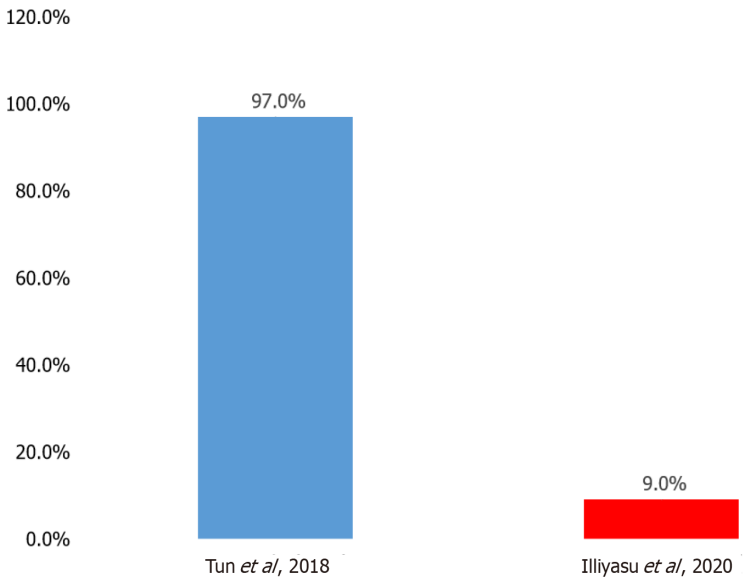
Strategies for enhancing uptake of HIVST in Nigeria

Studies conducted among young people in Nigeria by Obiezu-Umeh *et al*[32] and Iwelunmor *et al*[36] reported that the cost of HIVST was the strongest determinant for choosing HIVST services. The cost of HIVST ranged from 200 to 4000 Naira (approximately United States Dollar 0.55-11.07). However, the



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Figure 5 Acceptability of human immunodeficiency virus self-testing in Nigeria.



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Figure 6 Uptake of human immunodeficiency virus self-testing.

majority of young people suggested 500 Naira (approximately United States Dollar 1.38) as the preferred cost of the kit. Young people argued that the high cost of HIVST remained a major barrier to uptake since most young people might not be willing to purchase the kit given that HIVST kits are available in some hospitals and non-governmental organizations either free of charge or at a subsidized rate[32].

Ong *et al*[33] and Obiezu-Umeh *et al*[32] reported access location as a major driver and the most influential driver, respectively, of HIVST uptake. Obiezu-Umeh *et al*[32] reported privately-owned facilities, registered pharmacies, youth-friendly centres, supermarkets, and online stores as the most cited preferred locations to access HIVST kits. Ong *et al*[33] added that the least influential driver of HIVST uptake was the type of specimen needed for HIVST. Obiezu-Umeh *et al*[32] and Iwelunmor *et al*[36] suggested making HIVST more appealing to young Nigerians. This could be achieved by repackaging existing HIVST products with colours, taglines, designs, and youth-friendly animations[32,36]. Iwelunmor *et al*[36] also found that providing instruction for use translated into the three most common Nigerian languages (Igbo, Hausa, and Yoruba) would further enhance the appeal and uptake of the product among diverse segments of youths in Nigeria.

Studies among young people, MSM, and KOLs recommended using social media (Facebook/SMS and WhatsApp, *etc.*) and bulk SMS messages to enhance the uptake of HIVST[36,37]. In contrast, a quantitative study among key populations in Nigeria reported that 85% of female sex workers and 68% of MSM preferred an in-person modality of receiving information on HIVST services[37]. Furthermore, Sekoni *et al*[37] further suggested using KOLs to distribute and enhance the uptake of HIVST kits among MSM. Similarly, Iwelunmor *et al*[36] opined that recruiting local celebrities to join HIVST online campaigns and endorse HIVST-related hashtags could generate high demand for HIVST services and promote its uptake among their teeming fans who are mostly young people.

DISCUSSION

HIVST is a rapidly growing HIV testing strategy that is gradually gaining acceptability globally. However, the level of acceptability and strategies to enhance the uptake of HIVST varies across different populations and settings. In this systematic review, we examined the acceptability of HIVST and strategies to enhance the uptake of HIVST in Nigeria. Our findings revealed a high level of acceptability of HIVST in most of the studies included in this systematic review with many citing privacy and convenience as key factors in their willingness to use the service[5,7,15,27-31,38]. These findings are consistent with previous studies, which reported high acceptability and ease of use of HIVST in other settings such as United States, Tanzania, and Zimbabwe[39-41].

For instance, a recent cross-sectional study conducted in Tanzania among medical students showed a high level of knowledge, acceptability, and willingness to use oral fluid HIVST[39]. Similarly, a recent survey among people who use illicit drugs in the United States reported high acceptability of an HIVST program, and 77% of study participants were willing to use HIVST kits regularly if available[40]. In addition, a campus-based distribution of oral HIVST was highly acceptable among young adult students in Zimbabwe, with 97.1% of participants indicating willingness to use oral HIV self-tests[41]. However, some studies have reported low acceptability for HIVST among specific populations, such as MSM in Brazil, where less than half (47.3%) were willing to use HIVST[42].

Cost was a significant barrier to HIV testing among young people in Nigeria, and they preferred free or low-cost testing services[26]. The cost of HIVST in the private sector, especially in low-income contexts, may contribute to an unwillingness to use HIVST, which justifies the need for free distribution to key and priority groups in the public sector. Additionally, the fear of getting a positive result without being appropriately linked to a health service could also contribute to low acceptance of HIVST, thus emphasizing the importance of a peer navigator to support clients across the continuum of care.

The uptake of HIVST and linkage to care services varies across different population groups and countries. This systematic review found a high uptake of HIVST among MSM in Nigeria, similar to findings from Bangkok, Thailand[35,43], while low uptake of HIVST has been reported in South Africa and China[44,45]. This variation in findings could be attributed to differences in the level of awareness about HIVST among the studied populations. Factors such as education level, age, marital status, and knowledge about HIV can also influence the uptake of HIVST[27,28,35,44-46].

A study conducted in the Republic of Congo reported a high linkage to HIV care services (82.2%) among individuals with a reactive result from HIVST, which is consistent with the findings of another study in Nigeria[47]. However, studies have highlighted several barriers to linkage to care, such as social stigma, lack of communication about the benefits of testing, and the referral process after testing, as well as a lack of a supportive peer network to encourage linkage after testing[27,28,48]. Therefore, it is important to identify and address these barriers to linkage to care to ensure that HIVST programs are effective in reducing the burden of HIV and promoting early diagnosis and treatment.

Further research is needed to develop interventions that address the barriers to uptake and linkage to care. The systematic review on strategies to enhance HIVST uptake shows the importance of accessible points of HIVST distribution and involvement of young people in the development and design of HIVST services to address their preferences, which include privacy, confidentiality, convenience, and assurance of accuracy in order to enhance uptake[32,33,36]. These findings are supported by studies from Rwanda[49,50] which suggested that involving the target population in program design could improve HIVST uptake. The co-creation process that involved men in Rwanda identified the need for a comprehensive health education program to address barriers to HIVST uptake. Key stakeholders emphasized the need for community engagement, regulatory frameworks, and sustained political commitment to promote the increase of HIVST.

Furthermore, this systematic review also highlighted additional strategies to enhance HIVST uptake in Nigeria, including increasing awareness, regulating the sale of self-test kits, subsidizing the cost of self-test kits, maintaining consistent availability of self-test kits, and documenting HIVST standards and policies[35,38,51,52]. The articles also suggested that mobilization campaigns, training for people involved in implementation, and engaging key stakeholders such as religious and community leaders, employers, KOLs, celebrities, and health workers could accelerate HIV testing and promote uptake and linkage to care services.

Overall, the findings suggested that tailored communication strategies that address misinformation, misconceptions, and mistrust about HIVST and pre-exposure prophylaxis are needed for improved uptake of HIVST among key populations in Nigeria[38]. The involvement of stakeholders and the target population can lead to the design of HIVST programs that address the unique needs and preferences of each population, ultimately improving HIV testing and linkage to care services[49].

CONCLUSION

In summary, the landscape of HIVST in Nigeria is still in its infancy with a limited evidence base. Therefore, there is a compelling need for more high-quality research such as randomized clinical trials to advance our understanding of HIVST. This study revealed a shortage of implementation science research, despite the various self-testing activities ongoing in Nigeria. The investigators also noted lack of studies evaluating other HIVST distribution models, such as workplace, community distribution, and distribution among facility providers, and sub-populations, like pregnant women, people who inject drugs, and female sex workers. Only one study from Southern Nigeria evaluated programmatic HIVST distribution data among CPs. While the acceptability of HIVST is generally high in Nigeria when measured from the intention-to-use perspective, actual use in programmatic implementation was lower, primarily due to the cost barrier among pharmacy retail outlets. Therefore, innovative financing approaches targeting different population segments are necessary for effective scaling and growth of the HIVST market in Nigeria using demand side subsidy financing, total market approach, and social marketing.

More controlled implementation studies are required to test the acceptability of HIVST. The use of KOLs among key populations has been successful in increasing the acceptability and uptake of HIVST. The uptake of HIVST was generally high among reported studies, except for reported HIVST results. Therefore, more studies are needed to evaluate factors responsible for poor uptake of HIVST result retrieval and how uptake will compare in routine programs *vs* real-life settings in the absence of support and resources that enhance HIVST uptake. In conclusion, despite limitations, this is the first systematic literature review of HIVST in Nigeria, providing valuable insights into the evidence base on the acceptability and uptake of HIVST in the country.

ARTICLE HIGHLIGHTS

Research background

Nigeria has a high burden of human immunodeficiency (HIV)/acquired immune deficiency syndrome, and a significant proportion of infected individuals remain untested due to fear of stigma and discrimination. HIV self-testing (HIVST) is recommended by the World Health Organization as a tool for improving testing uptake and achieving the United Nations Programme on HIV and acquired immune deficiency syndrome 90-90-90 target. However, HIVST is still a growing intervention in Nigeria, and there is a need to systematically review its acceptability and uptake in the country.

Research motivation

To systematically review the acceptability, existing regulations, and strategies for enhancing the uptake of HIVST in Nigeria.

Research objectives

To fill a crucial gap in understanding the HIVST landscape in Nigeria and provide insights into the evidence base on the acceptability and uptake of HIVST in the country.

Research methods

A systematic literature review was conducted, and 18 articles were included in the analysis.

Research results

The study found that the acceptability of HIVST is generally high in Nigeria from the intention-to-use perspective. However, the actual use of HIVST in programmatic implementation was lower than expected. The study recommends more controlled implementation studies to test the acceptability of HIVST and to explore factors responsible for poor uptake. The use of key opinion leaders among key populations has been found to be successful in increasing the acceptability and uptake of HIVST. However, cost remains a major barrier to the acceptability of HIVST among pharmacy retail outlets.

Research conclusions

The present study provided crucial understanding of the HIVST landscape in Nigeria, which is young

and evolving. The study highlighted the need for further high-quality research in this area and recommended innovative financing approaches targeting different population segments for effective scaling of HIVST under the total market approach.

Research perspectives

More studies are required to evaluate how the uptake of HIVST compares in routine programs *vs* real-life settings in the absence of support and resources that enhance HIVST uptake. Overall, this study contributed to the current knowledge base on HIVST in Nigeria and highlighted the need for further high-quality research in this area.

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FOOTNOTES

Author contributions: Adepoju VA and Adelekan A conceived the study; Onoja AJ provided overall guidance to the study; Adelekan A, Umebido C, and Adepoju VA conducted screening and led data extraction; Adelekan A and Onoja AJ drafted the manuscript; All authors read and approved the final version.

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Country/Territory of origin: Nigeria

ORCID number: Victor Abiola Adepoju 0000-0001-8379-1001; Ademola Adelekan 0000-0001-5729-6824.

S-Editor: Liu XF
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P-Editor: Ju JL

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SYSTEMATIC REVIEWS

Preferences for oral- *vs* blood-based human immunodeficiency virus self-testing: A scoping review of the literature

Victor Abiola Adepoju, Winifred Imoyera, Ali Johnson Onoja

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Victor Abiola Adepoju, Winifred Imoyera, Department of HIV and Infectious Diseases, Jhpiego Nigeria, an affiliate of John Hopkins University, Abuja 900901, Federal Capital Territory, Nigeria

Ali Johnson Onoja, Research, African Health Project, Abuja 900901, Federal Capital Territory, Nigeria

Corresponding author: Victor Abiola Adepoju, MD, self-testing in Africa Project Director, Department of HIV and Infectious Diseases, Jhpiego Nigeria, an affiliate of John Hopkins University, Plot 971, Rueben Okoya Street, Abuja 900901, Federal Capital Territory, Nigeria. schrodinga05@yahoo.com

Abstract

BACKGROUND

The evidence on preferences for oral- *vs* blood-based human immunodeficiency virus self-testing (HIVST) has been heterogenous and inconclusive. In addition, most evaluations have relied on hypothetical or stated use cases using discreet choice experiments rather than actual preferences among experienced users, which are more objective and critical for the understanding of product uptake. Direct head-to-head comparison of consumer preferences for oral- versus blood-based HIVST is lacking.

AIM

To examine the existing literature on preferences for oral- *vs* blood-based HIVST, determine the factors that impact these preferences, and assess the potential implications for HIVST programs.

METHODS

Databases such as PubMed, Medline, Google Scholar, and Web of Science were searched for articles published between January 2011 to October 2022. Articles must address preferences for oral- *vs* blood-based HIVST. The study used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist to ensure the quality of the study.

RESULTS

The initial search revealed 2424 records, of which 8 studies were finally included in the scoping review. Pooled preference for blood-based HIVST was 48.8% (9%-78.6%), whereas pooled preference for oral HIVST was 59.8% (34.2%-91%) across all studies. However, for male-specific studies, the preference for blood-based

HIVST (58%-65.6%) was higher than that for oral (34.2%-41%). The four studies that reported a higher preference for blood-based HIVST were in men. Participants considered blood-based HIVST to be more accurate and rapid, while those with a higher preference for oral HIVST did so because these were considered non-invasive and easy to use.

CONCLUSION

Consistently in the literature, men preferred blood-based HIVST over oral HIVST due to higher risk perception and desire for a test that provides higher accuracy coupled with rapidity, autonomy, privacy, and confidentiality, whereas those with a higher preference for oral HIVST did so because these were considered non-invasive and easy to use. Misinformation and distrust need to be addressed through promotional messaging to maximize the diversity of this new biomedical technology.

Key Words: Human immunodeficiency virus self-testing; Preferences; Oral human immunodeficiency virus self-testing; Blood-based human immunodeficiency virus self-testing

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Core Tip: We conducted a scoping review of the literature to determine the preferences for oral- *vs* blood-based human immunodeficiency virus self-testing (HIVST) and related factors. We searched PubMed, Medline, Google Scholar, and Web of Science databases for articles published between January 2011 and October 2022 that addressed preferences for oral- *vs* blood-based HIVST. The pooled preferences for blood- and oral-based HIVST were 48.8% and 59.8%, respectively. For male-specific studies, the preference for blood-based HIVST was higher than for oral. These results highlight the need to address misinformation and distrust through promotional messaging to maximize the diversity of this new biomedical technology.

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INTRODUCTION

The Joint United Nations Programme on human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) (UNAIDS) has set 95:95:95 as a strategy to end HIV/AIDS by 2030. Although much progress has been made in achievement of the first 95 (*i.e.* 95% of individuals with HIV should test and know their HIV status), progress has been slow among hard-to-reach populations such as men, key populations, adolescents, and young persons. Men living with HIV perform less than women, with only 82% of men living with HIV knowing their HIV status[1]. Compared to women living with HIV, there are 740000 more men living with HIV who do not know their HIV status, 1.3 million more men who are not on treatment, and 920000 more men who are not virally suppressed[2]. The World Health Organization (WHO) released the first normative guideline on HIV self-testing (HIVST) in 2016[3]. WHO recommended HIVST as an additional approach to HIV testing services and recently added that both oral- and blood-based options should be provided. HIVST is safe, private, confidential, and convenient with the potential to improve access to testing for hard-to-reach populations such as men, adolescents, and young people as well as key populations. ST, being the first step in the care continuum, presents an enormous opportunity to close the HIV testing gap and achieve the global 95:95:95 fast track target set by UNAIDS. Self-testing empowers consumers to control when, where, and how they test for any of these diseases. Given the challenges in accessing traditional, provider-led testing services such as long distance from facilities, limited operating hours of conventional clinics, competing client priorities such as job and schooling, stigma, high cost, poor awareness, and dearth of culturally competent healthcare workers[4,5], ST as an alternative testing model, is a useful tool to expand access to testing for HIV, especially among vulnerable groups.

As of August 2022, six HIVST have been prequalified by the WHO (one using oral fluid and five using whole blood), *i.e.* Wondfo, Mylan, Insti, Check Now, Sure Check, and OraQuick[6,7]. However, evidence on preferences for oral- *vs* blood-based options has been heterogenous and inconclusive[8,9]. In addition, most evaluations have relied on hypothetical or stated use cases using discreet choice experiments[10,11] rather than actual use preferences from experienced end-users, which are more

objective and critical for uptake. Two main types of HIVST are available: oral- and blood-based tests. While both tests have demonstrated high sensitivity and specificity, the preferences of individuals for one test type over the other remain unclear. Understanding these preferences is crucial to promoting the widespread adoption and usage of HIVST.

The purpose of this scoping review is to provide a comprehensive overview of the literature on preferences for oral- *vs* blood-based HIVST, identify factors influencing these preferences, and explore the implications of these preferences for the promotion and implementation of HIVST programs. By synthesizing existing evidence, this review aims to inform policy-makers, healthcare providers, and other stakeholders involved in the design and implementation of HIVST programs, in order to maximize uptake and improve overall public health outcomes.

MATERIALS AND METHODS

The scoping review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Figure 1). These reviews follow explicit, pre-specified and reproducible methods in order to identify, evaluate, and summarize the findings of all relevant individual studies (Grant and Booth, 2009)[12].

Search strategy

One of the authors (Adepoju VA) searched for eligible studies between October 15 and 20, 2022. The Arksey and O'Malley[13] (2005) methodological framework guided the scoping of the published data. The scoping review conducted in this study was not registered in a registry such as PROSPERO. We chose not to register this scoping review, as registration is not a mandatory requirement for scoping reviews and our primary aim is to provide a broad overview of the literature rather than conduct a systematic assessment of the evidence. Although the Reference Citation Analysis tool was available for use, it was not utilized for this review. This decision was made based on the nature of the research question and the inclusion and exclusion criteria developed for the review, which ensured that all relevant studies were identified through the comprehensive search strategy described above.

Data sources

Searched databases included PubMed, MEDLINE, Web of Science, and Google Scholar. For studies that may have been missed in the electronic search, we used reference lists of all the articles identified for cross-referencing. The first search took place between October 1 and 6, 2022, whereas the second took place between October 8 and 14, 2022. Detailed inclusion and exclusion criteria were developed with caution, to make sure that they matched the review questions and involved sufficient details to help point out all relevant studies and exclude irrelevant ones. The researchers then embarked on a two-stage process in which two reviewers independently screened the titles and abstracts for eligibility to be included in the final selection of papers. A combination of terms was used in the database searches; specifically: "HIV self-testing," OR "HIVST" OR "HIV self-screening" OR "HIVSS" AND "preferences" AND "values" AND "oral- and blood-based" OR "oral and fingerstick HIVST" OR Oral and capillary" OR "oral- *vs* blood-based." Specific keywords were combined with Boolean operators in the literature search (Table 1).

Study selection

The systematic searches for eligible articles retrieved 2424 studies and 1454 duplicates were eventually removed. The authors (Adepoju VA, Imoyera W) independently screened the titles and abstracts for eligibility with the condition that if one or both authors identified the article as relevant, then the full-text review would be carried out. The researchers solved any disagreements *via* discussions and reached a consensus. After the title and abstract screening, two reviewers (Adepoju VA, Imoyera W) independently screened the full text of selected articles. Disagreements were resolved through discussions with a third reviewer (Onoja AJ) for final inclusion. The articles were selected in several parts, which allowed the reviewers to have a regular discussion of the eligibility criteria, ensuring the same understanding of the criteria, and the criteria remaining the same throughout the article selection phase. The researchers did not assess the risk of bias of the included studies. As in many scoping reviews, the goal was to describe preferences for oral- *vs* blood-based HIVST.

Inclusion and exclusion criteria

Studies published in peer-reviewed journals between January 2011 and October 2022 and focusing on preferences of oral- *vs* blood-based HIVST among actual users were reviewed.

Inclusion criteria were: primary studies with participants aged 15 years or more with no geographic or population limitations; studies reporting on user preferences for HIVST with only two comparison groups (*i.e.* oral- *vs* blood-based HIVST); studies that adapted HIV Point of Care for HIVST for research purpose only; and studies that included actual users of oral- and blood-based HIVST. Exclusion criteria were studies comparing either oral- or blood-based HIVST with facility-based test or any other HIV

Table 1 Search terms used in the literature search on preference for oral- *vs* blood-based human immunodeficiency self-testing in Nigeria

Search terms	OR	AND	AND
HIVST	HIV self-testing	HIV self-screening	
HIV self-testing			
HIVSS			HIV self-screening
HIV self-screening			
Blood-based HIVST	Fingerstick HIVST		Capillary HIVST
Fingerstick HIVST			
Capillary HIVST			Oral HIVST
Oral HIVST			
Preference			

Please note that the terms in the "OR" column are combined with an "OR" operator, whereas the terms in the "AND" columns are combined with an "AND" operator. The search strategy is designed to identify studies focusing on preferences for different HIV self-testing methods within the Nigerian context. HIV: Human immunodeficiency virus; HIVSS: HIV self-screening; HIVST: HIV self-testing.

testing approaches (*e.g.*, Voluntary Counselling and Testing, mail-in, Dry Blood Sample); studies evaluating user preferences for one type of HIVST only (*i.e.* oral- or blood-based specimen); studies where comparison group for preferences was not clear, not stated or measured qualitatively; and studies including hypothetical users rather than actual users of HIVST. Also excluded were articles published before January 2011, conference papers, books, studies with no full-text available, and magazines. This is because HIVST was not popular before this period and publications on this subject matter were either scarce or non-existent before this period. In accordance with PRISMA guideline 16b, we have cited and explained the exclusion of studies that appeared to meet the inclusion criteria but were ultimately excluded. The reasons for their exclusion are provided in the results and appendix section (Supplementary Table 1) ensuring transparency in the review process.

Data extraction

The authors extracted relevant data using a standard excel-based template. Two authors (Adepoju VA, Imoyera W) independently extracted the data, and the results were reviewed and verified by both authors for quality and clarity. Two authors (Adepoju VA, Onoja AJ) separately and independently assessed the full text of the potentially eligible publications. Disagreements were resolved by consensus. Initial agreement was obtained on 90% of the items, and discrepancies were discussed between authors until 100% agreement was obtained. The following information was extracted from the included studies: author name and year of study, study design, type of specimen, product type, population and age, prevalence of preference for oral- and blood-based HIVST and major findings (Table 2). After extracting relevant information from the studies, the authors constructed a more specific classification for preferences of oral- *vs* blood-based HIVST.

List of papers reviewed

The search results are shown in Figure 1, along with a summary of the papers consulted (the PRISMA flow chart). Although 2424 research articles were retrieved initially from the databases, only 8 met the inclusion criteria for this scoping review.

RESULTS

During the study selection process, we identified several studies that initially appeared to meet our inclusion criteria but were ultimately excluded upon closer examination and based on the predefined inclusion and exclusion criteria. We have provided a comprehensive list of these excluded studies and the reasons for their exclusion in the Supplementary Table 1. By documenting this information, we aim to ensure transparency and reproducibility in our review and study selection process and to demonstrate compliance with PRISMA 16B.

Geographic and population distribution of the included articles

The total number of participants across the 8 studies was 7129 (40-4496). Of the eight studies reviewed,

Table 2 Summary of the included studies, *n* = 8

Ref.	Country	Study design	Type of specimen	Product type	Population, age in yr	Preference for oral, %	Preference for blood, %	Other findings
Tonen-Wolyec *et al*[14], 2020	The Republic of Congo	Cross-sectional	Oral *vs* Fingerstick	Oraquick, Exacto	General population,18-49	85.6	78.6	Comparable accuracy. University education and higher risk increases BB preference
Trabwongwitaya *et al*[15], 2022	Thailand	Cross-sectional	Oral *vs* Fingerstick	Oraquick, INSTI	Young adult KP,18-24	34.4	65.6	Performance and interpretation, O-93.3%, 100%; B-89.5%,98%
Cassell *et al*[19] , 2022	Cambodia	Cross-sectional	Oral *vs* Fingerstick	Oraquick, CombokitsAbbot	KPs, 15+	88.5	11.5	Assisted-98.6%; Unassisted-1.4%
Shapiro *et al*[20], 2020	South Africa	Cross-sectional	Oral *vs* Fingerstick	Oraquick, Atomo	Adult men, 18+	42	58	10% and 90% will prefer different and the same kit for repeat tests, respectively
Lippman *et al* [17], 2018[1]	South Africa	Cross-sectional	Oral *vs* Fingerstick BB	Oraquick, Atomo	MSM	34.2	64.6	97% will use HIVST again if available in the future
Lee *et al*[16], 2022	Australia	Randomized Clinical Trial (RCT)	Oral *vs* Fingerstick	Oraquick, Atomo	MSM,18+	41	58	O-not swabbing both gum, placing buffer on stand; BB-filling test channel, squeezing finger for blood drop
Ritchwood *et al* [18], 2019	South Africa	Qualitative	Oral *vs* Fingerstick	Not stated	Young adult,18-24	80	20	Post-test opinion change on ease of use and trust in result
Gaydos *et al*[21], 2011	United State	Crosssectional	Oral *vs* Fingerstick	Oraquick, Unigold	Emergency department, 18-64	91	9	'Trust in result' O-similar for initial HCW-led and client ST (91%); B-client BBST result more (91.7%) than HCW provided HIV test (77.8%)

[1]Indicates that it is a subsequent publication by the same author(s) in the same year. B: Blood; BBST: Blood-based self-testing; HCWs: Healthcare workers; HIV: Human immunodeficiency; KP: Key Population; MSM: Men who have sex with men; O: Oral; ST: Self-testing.

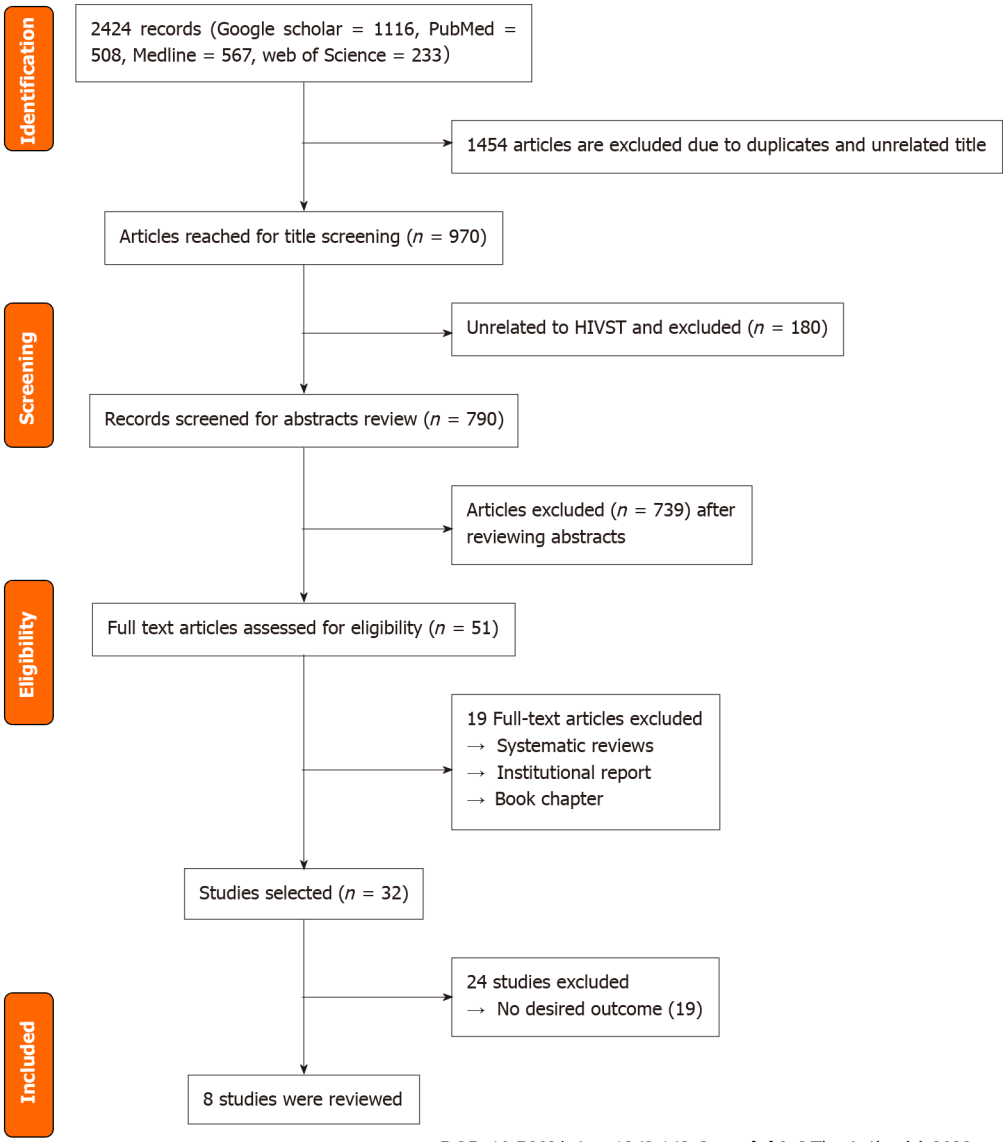
three studies were from South Africa and one each was from Cambodia, the United States, Thailand, Australia, and the Democratic Republic of Congo Figure 2. Three studies involved the general population (*n* = 3)[14-16], four involved the key population (*n* = 4)[14-17], and one involved young people (*n* = 1)[18]. A total eight studies, *i.e.* 6 quantitative studies[14,15,17,19-21], 1 randomized control trial[16], and 1 qualitative[18], were included in the study.

Year of publication of included studies

Out of the eight articles included, three were published in 2022[15,16,19], two in 2020[14,20], one in 2018 [17], one in 2019[18], and one in 2011[21] (Figure 3).

Preference for oral- vs blood-based HIVST

One hundred percent of the studies reported preference based on the actual use of HIVST, and 50% reported usability. Four of the eight studies (50%) reported a higher preference for blood-based HIVST [16,18-20], whereas four of the eight studies (50%) reported a higher preference for oral HIVST[14,15,17, 21]. Pooled preference for blood-based HIVST was 48.8% (9%-78.6%), whereas pooled preference for oral HIVST was 59.8% (34.2%-91%) across all studies. However, for male-specific studies[16,18-20], preference for blood-based HIVST (58%-65.6%) was higher than that for oral (34.2%-41%). The four studies that reported a higher preference for blood-based HIVST were in men, and participants considered blood-based HIVST to be more accurate and rapid whereas studies reporting higher preference for oral HIVST did so because they were considered non-invasive and easy to use with few false-negative results.



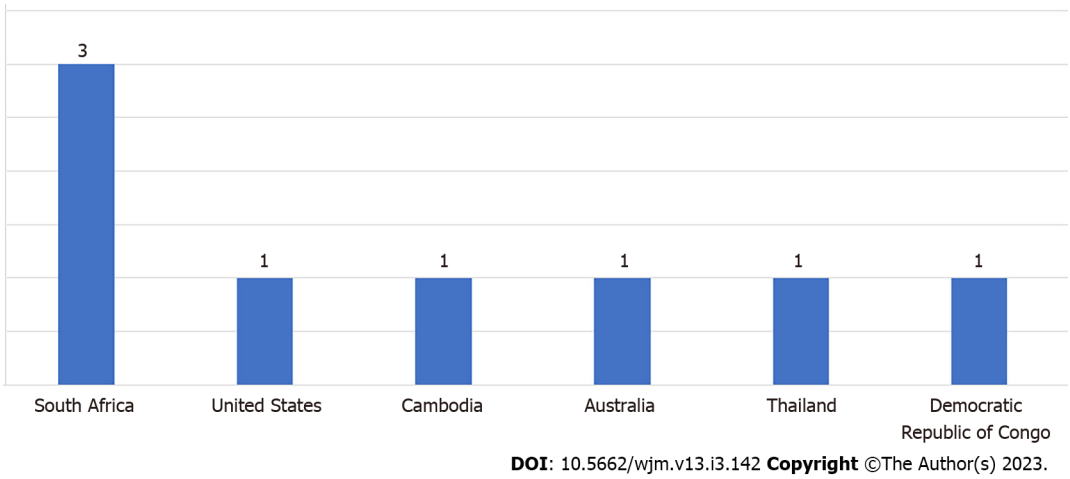
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Figure 1 Preferred reporting items for systematic reviews and meta-analyses flowchart: Study selection process for the scoping review on preferences for oral- *vs* blood-based human immunodeficiency virus self-testing.

DISCUSSION

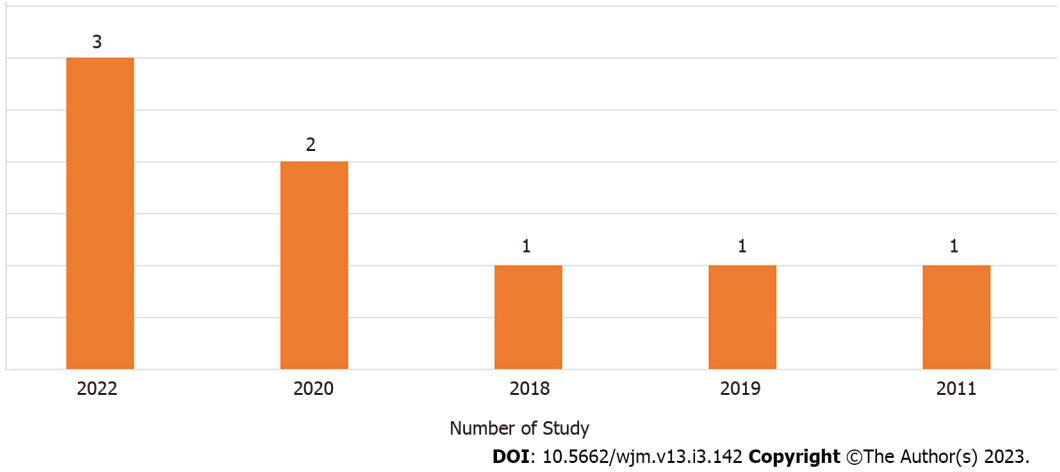
Overall, the study observed a slightly higher preference for oral than fingerstick HIVST. Similar to this finding, in studies among pregnant women in India[22], primary healthcare attendees in South Africa [23], female sex workers in China[24], and young people in Nigeria[25], participants who chose oral HIVST (over blood-based) cited ease of use and ability to avoid needle prick as reasons for choosing oral HIVST. Those who did not choose oral HIVST distrusted its capacity to detect HIV in saliva specimens. The distrust in HIV detection in saliva may have stemmed from HIV messaging that has historically emphasized that HIV can neither be acquired nor transmitted through kissing and oral sex[26,27]; hence, clients have questioned the scientific basis for HIV detection in oral fluid.

Furthermore, a significantly higher preference for blood-based HIVST than oral HIVST was noted in male-specific studies in this scoping review. Consistent with this finding, preferences for blood-based HIVST in men who have sex with men (MSM) in the United Kingdom and heterosexual men in Singapore were higher due to its accuracy, rapidity of results, and minimal false-negative results[28-30]. Preferences were also associated with certain factors such as previous testing, type of product used for recent testing, and presence of high-risk sexual behavior, indicating that these factors may influence individual preferences[31-33]. For instance, a study previously highlighted that individuals reporting recent high-risk sexual behaviors (*e.g.*, unprotected sex, sex when drunk) were less likely to use oral HIVST[32], whereas the likelihood of using blood-based HIVST increased when offered with information on other sexually transmitted infections[33]. Men's greater preference for blood-based HIVST was influenced by perceived higher risk, desire for accuracy, and perception of having lesser



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Figure 2 Number of included studies by country.



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Figure 3 Number of included studies by year of publication.

false-negative results[30]. Previous studies have also suggested that the accuracy of blood-based self-tests is higher than that of oral-fluid self-tests due to the lower quantity of HIV antibodies in oral fluid compared with whole blood[34] and reduced sensitivity for oral fluid testing for antibody detection (compared with blood testing) when specimen was obtained early after HIV infection[35]. Moreover, evaluation report of the third-generation blood-based HIVST showed very high sensitivity of 100% and high specificity of 99.9% and the ability of this product to detect HIV infections 7 d sooner than second-generation tests (*i.e.* from day 21 of infection instead of 28 d associated with most second-generation oral- and blood-based HIVST)[36]. One would expect usability of blood-based testing to be a major barrier, especially among men where preference was high. By contrast, a usability index average of 92.8% (92.2%-95.5% for oral HIVST; 84.2%-97.6% for blood-based HIVST) was reported in a study that evaluated the usability of seven WHO Prequalified HIVST kits (five blood-based and two oral HIVST) in South Africa[37]. Since both oral- and blood-based HIVST are complementary, a choice-based approach is therefore needed to optimize HIV testing programs and close the gaps between HIV testing and treatment.

There are several limitations to consider when interpreting our findings. First, we only used four databases to search the literature and may have missed articles not embedded. That notwithstanding, these databases are the basic sources of public health literature. Also, by not including conference abstracts, more recent unpublished articles may have been missed. Moreover, by reviewing citations of scoping and systematic reviews, the chances of incorporating the full breadth of the research through our search strategies were increased. We are convinced of having reached saturation with our methods. The real strength of the study lies in the inclusion of studies that offered both oral- and blood-based HIVST to actual users in real-world situations rather than experimental studies. This has removed the generalizability bias often seen in studies that offer only one type of HIVST or measure preferences from an intention-to-use perspective[38,39].

CONCLUSION

The scoping review consistently showed that men preferred blood-based HIVST than oral HIVST due to a higher risk perception and desire for a test that provides higher accuracy coupled with autonomy, rapidity, privacy, and confidentiality. The UNAIDS 2021 report showed a huge gap in knowledge of HIV status among general men and MSM, whereas AIDS-related death was higher in men than women due to late diagnosis, hence providing a blood-based HIVST option that can facilitate acceptability and the earlier diagnosis of HIV in men.

Similarly, the scoping review highlighted the diversity in preferences for oral- and blood-based HIVST and found that a single type of self-test kit is unlikely to cater for the preferences of diverse population and achieve high testing coverage. Integrating novel biomedical instruments into standard clinical and community procedures can occasionally prove difficult, as evidenced by the adoption of oral and injectable preexposure prophylaxis along with contemporary contraceptive methods. That notwithstanding, Ministries of Health and country programs should consider both blood and oral HIVST options. Offering choices among multiple kits may be the best way to maximize uptake and reach populations who may not otherwise test for HIV. Offering broader choices for HIVST could have a greater impact on testing uptake, but more research is needed to address misconceptions that drive HIVST and identify effective, population-specific dissemination channels needed to promote HIVST choices so people can make appropriately informed choices.

ARTICLE HIGHLIGHTS

Research background

Human immunodeficiency virus self-testing (HIVST) has been shown to increase testing rates and improve early HIV diagnosis. However, there are different testing modalities, including oral- and blood-based HIVST, and little is known about the preferences for these different types of HIVST.

Research motivation

Identifying preferences for oral- *vs* blood-based HIVST is crucial for the development and implementation of effective HIVST programs. Understanding the factors that influence these preferences can also inform strategies for increasing uptake of HIVST.

Research objectives

The main objective of this scoping review was to provide a comprehensive overview of the literature on preferences for oral- *vs* blood-based HIVST. Specific objectives included identifying factors that influence preferences, exploring the implications of these preferences for the promotion and implementation of HIVST programs, and highlighting gaps in the literature.

Research methods

A scoping review methodology was used to identify and synthesize relevant literature on preferences for oral- *vs* blood-based HIVST. The review included studies published in English between 2011 and 2021 that focused on actual and not hypothetical users of HIVST.

Research results

The search yielded 2424 records, of which 8 studies were included in the review. Across all studies, pooled preference for oral HIVST was 59.8%, whereas for blood-based HIVST, it was 48.8%. However, in studies specific to men, the preference for blood-based HIVST (58%-65.6%) was higher than oral (34.2%-41%). Men favored blood-based HIVST because of its perceived accuracy and rapidity, whereas oral HIVST was preferred for being non-invasive and easy to use.

Research conclusions

Preferences for oral- *vs* blood-based HIVST are influenced by various factors, including user characteristics such as sex, testing context, and perceived test accuracy. Programs promoting HIVST should consider these factors when designing and implementing HIVST programs. Further research is needed to explore the impact of these preferences on HIV testing rates and to identify effective strategies for increasing the uptake of HIVST.

Research perspectives

Future research should focus on identifying effective strategies for increasing the uptake of HIVST, particularly among populations that may have unique preferences or barriers to testing. Longitudinal studies could also help to explore the impact of these preferences on HIV testing rates and linkage to care. Additionally, studies should continue to explore the accuracy and feasibility of new HIVST techno-

logies.

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FOOTNOTES

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Country/Territory of origin: Nigeria

ORCID number: Victor Abiola Adepoju 0000-0001-8379-1001.

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META-ANALYSIS

Microvessel density in patients with gastrointestinal stromal tumors: A systematic review and meta-analysis

Konstantinos Perivoliotis, Ioannis Baloyiannis, Athina A Samara, Prodromos Koutoukoglou, Panagiotis Ntellas, Katerina Dadouli, Maria Ioannou, Konstantinos Tepetes

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Konstantinos Perivoliotis, Ioannis Baloyiannis, Athina A Samara, Konstantinos Tepetes, Department of Surgery, University Hospital of Larissa, Larissa 41110, Greece

Prodromos Koutoukoglou, Katerina Dadouli, Research Methodology in Biomedicine, Biostatistics and Clinical Bioinformatics, University of Thessaly, 41110 41110, Greece

Panagiotis Ntellas, Maria Ioannou, Department of Pathology, University Hospital of Larissa, Larissa 41110, Greece

Corresponding author: Athina A Samara, MD, Surgeon, Department of Surgery, University Hospital of Larissa, Mezourlo Hill, Larissa 41110, Greece. at.samara93@gmail.com

Abstract

BACKGROUND

Gastrointestinal stromal tumors (GISTs) are considered the most common mesenchymal tumors of the gastrointestinal tract. Microvessel density (MVD) constitutes a direct method of vascularity quantification and has been associated with survival rates in multiple malignancies.

AIM

To appraise the effect of MVD on the survival of patients with GIST.

METHODS

This study adhered to Systematic reviews and Meta-Analyses guidelines and the Cochrane Handbook for Systematic Reviews of Interventions. Electronic scholar databases and grey literature repositories were systematically screened. The Fixed Effects or Random Effects models were used according to the Cochran Q test.

RESULTS

In total, 6 eligible studies were identified. The pooled hazard ratio (HR) for disease free survival (DFS) was 8.52 (95%CI: 1.69-42.84, $P = 0.009$). The odds ratios of disease-free survival between high and low MVD groups at 12 and 60 mo did not reach statistical significance. Significant superiority of the low MVD group in terms of DFS was documented at 36 and 120 mo (OR: 8.46, $P < 0.0001$ and OR: 22.71, $P = 0.0003$, respectively) as well as at metastases rate (OR: 0.11, $P = 0.0003$).

CONCLUSION

MVD significantly correlates with the HR of DFS and overall survival rates at 36

and 120 mo. Further prospective studies of higher methodological quality are required.

Key Words: Vascularity; Microvessel density; Gastrointestinal stromal; Survival; Meta-analysis

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Core Tip: This systematic review and meta-analysis summarize all available data regarding the prognostic role of microvessel density (MVD) in gastrointestinal stromal tumors (GISTs). MVD measurement affects long term GIST survival. However, further prospective studies are necessary.

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INTRODUCTION

Gastrointestinal stromal tumours (GISTs) are the most common mesenchymal tumours of the gastrointestinal (GI) tract. According to existing literature, the average GIST incidence is estimated at 10 to 15 cases per million, ranging from 4.3 to 22/1000000 between different geographical locations[1,2]. Furthermore, although the age of reported cases spans from 10 to 100 years, the median GIST presentation appears during the mid-60 years of age, with no discrepancies in terms of gender allocation [1,2].

Based on recent studies, the origin of these tumours can be traced to the interstitial cell of Cajal, a myenteric plexus pacemaker[2-4]. The most frequent GIST locations are the stomach (55.6%), small (31.8%) and large intestine (6%). Further primary sites include the oesophagus, the omentum, the mesentery and the retroperitoneum[1-2,5]. Regarding morphological characteristics, GISTs are classified in spindle cell, epithelioid cell and mixed type histological subgroups[6].

The majority of GISTs have been found to express KIT, a proto-oncogene protein[7]. Specifically, KIT or c-kit is positive through immunohistochemical staining in almost 95% of all GISTs[6], while KIT-negative GISTs have been demonstrated to harbour mutations of platelet-derived growth factor receptor-alpha[6,8,9]. Alteration of the function of these receptor tyrosine kinases is considered of major importance in the GIST oncogenesis, through the RAS-RAF-MAPK and PI3K-AKT-mTOR pathways[6]. Surgical excision is considered the gold standard treatment for GISTs. However, kinase inhibitor adjuvant therapy (*i.e.* imatinib and sunitinib) has been introduced for treatment of advanced and metastatic disease[10-15], improving the overall survival (OS) and time to progression rates. Despite this, treatment resistance and disease recurrence rates still remain a significant problem[11,13].

To prognose the therapy outcomes, various risk grading systems have emerged, including those proposed by Fletcher *et al*[16] and Miettinen *et al*[17]. Several clinical and histopathologic factors been investigated such as tumor size, mitotic activity, anatomical origin, tumor rupture, tumor mutation type, predominant cell type, cellular density, p53, Ki-67, neutrophil to lymphocyte ratio and blood vessel invasion[6,11,13,18-20].

Microvessel density (MVD) assessment technique, based on the original work of Weidner *et al*[21], constitutes a direct method of vascularity quantification, since it represents the number of small blood vessels in tumoral tissue. Estimation of the vasculature is achieved through the application of various immunohistochemical endothelium labelling stains, such as cluster of differentiation (CD) 31, CD34, CD105 and von Willebrand Factor (vWF). The correlation between tumoral MVD and overall survival outcome in GIST patients has been extensively researched[8-9,22-25]. However, to the best of our knowledge, there is still no study assessing overall prognostic value of MVD in these neoplasms.

Considering the above, a systematic literature review and meta-analysis of the reported outcomes was designed to estimate the pooled effect of tumor vascularity on survival of GIST patients, based on MVD measurements.

MATERIALS AND METHODS

Study protocol

The present meta-analysis was conducted based on the Cochrane Handbook for Systematic Reviews of

Interventions and Systematic reviews and Meta-Analyses (PRISMA) guidelines[26]. The study was not registered in current electronic databases.

Primary endpoint

The primary endpoint of the present meta-analysis was considered the Hazard Ratio (HR) of Disease-Free Survival (DFS) between low and high MVD measurements in patients suffering from GISTS. Pooled HR > 1 denoted a higher risk of death in patients with high MVD, compared to patients with low MVD.

Secondary endpoints

The secondary endpoints included pooled odds ratios (ORs) of DFS between high and low MVD measurements, at four specific time points (12, 36, 60 and 120 mo) of follow-up. Moreover, the pooled OR between high and low MVD tumours of the presence of metastases in GIST patients was estimated. A pooled OR > 1 suggested superiority of low MVD tumours when compared to respective high MVD tumours, in terms of survival endpoints. On the contrary, concerning the metastases endpoint the opposite applied.

Eligibility criteria

Eligible studies were prospective or retrospective trials with a study population consisting of GIST patients, whose outcomes of interest were reported in English and were retrievable. Specifically, the study design must have incorporated a primary tumor MVD assessment.

Exclusion criteria consisted of studies written in a language other than English, with no endpoint of interest, insufficient survival data and no human studies. Furthermore, studies in the format of a letter, conference abstract, expert opinion or duplicate trials were not incorporated in the meta-analysis.

Literature search

A systematic literature search in electronic scholar databases (MEDLINE, CENTRAL, Scopus and Web of Science) and grey literature repositories (OpenGrey.eu and medRxiv) was performed to identify eligible studies. The last search date was December 2022. The literature search included the following search keywords: 'gist', 'gastrointestinal stromal tumor', 'stromal tumor', 'mvd', 'microvessel density' and 'microvascular density'.

Study selection and data collection

The first step of screening included removing duplicate entries. Subsequently, titles and abstracts of the remaining studies were assessed based on the inclusion criteria. A full text review of accepted entries was then performed, to validate consistency with the eligibility criteria. The electronic database screening, study selection, data extraction as well as methodological and quality assessment were performed in duplicate and blindly by two independent investigators (K.P. and P.K.). To reach consensus, disagreements were resolved by mutual revision and discussion. If discrepancies were not resolved, the opinion of a third investigator (K.D) was considered.

The Newcastle-Ottawa Scale (NOS)[27] was utilized to perform rigorous quality and methodological evaluation of eligible studies. NOS evaluates non-RCT trials in certain endpoints, such as selection and comparability of study groups and confirmation of the exposure. Each included study was rated with a score ranging from 0 to 9. Cohen's k statistic was also calculated.

Statistical analysis

Data analysis and statistical computations were performed using Cochrane Collaboration RevMan version 5.3 and IBM SPSS version 23. The primary and secondary endpoints were reported in the form of HR and OR, respectively. Results of the analyses were presented with the corresponding 95% Confidence Interval (95%CI).

If eligible trials did not directly provide the HR or OR in the article results, they were estimated based on the algorithm proposed by Parmar *et al*[28] and Tierney *et al*[29]. Specifically, required data for the meta-analysis of trials endpoints were reconstructed from the Kaplan-Meier curves provided[30]. The precision of extracted coordinates was enhanced through utilization of a digitizing software (Digitizelt)[31].

If included trials did not provide the mean and standard deviation (SD) of continuous variables, they were estimated from the median and the Interquartile Range (IR) based on the algorithm described by Hozo *et al*[32]. Given a sample size of >25, the mean was considered equal to the median. For a sample of < 70, the SD was regarded as IR/4. In the other case, the SD was calculated as IR/6.

The statistical method applied was Mantel-Haenszel (MH) and the Inverse Variance for OR and HR, respectively. Both the Fixed Effects and Random Effects (RE) model were calculated. The final model that was estimated was based on the Cochran Q test. If statistically significant heterogeneity was present (Q test $P < 0.1$), then the RE model was applied. A further quantification of the heterogeneity was performed through the calculation of I^2. Statistical significance was considered at the level of $P < 0.05$.

Risk of bias across studies

To estimate the publication bias of included studies, the funnel plot of the primary outcome was visually inspected. Regarding the primary outcome, Egger's test was also performed.

RESULTS

Study selection

Through the above-mentioned search algorithm (Figure 1), 994 citations were retrieved (MEDLINE: 412, Web of Science: 526, Scopus: 31, CENTRAL: 1, OpenGrey.eu: 15, medRxiv: 9). The next step included the removal of 340 duplicate records. A total of 654 records underwent title and abstract screening, resulting in the exclusion of 631 entries (17 reviews/ meta-analyses, 2 conference abstracts, 4 paediatric studies, 608 irrelevant records). Examination of compliance with eligibility criteria extended to the full text articles of the previously accepted records. In total, 5 studies with inadequate survival data and 12 irrelevant studies were excluded. Subsequently, 6 studies[8-9,22-25] were included in the present meta-analysis.

Study characteristics

Table 1 summarizes the characteristics of included studies. Regarding study type, all trials had a retrospective design. Furthermore, all except one trial[9] were single centre, with sample size ranging from 53 to 124. More specific information regarding the analysis and total specimen sample are reported in Table 1. Mean patient age and gender allocation are also displayed in Table 1. Mean follow-up period extended from 2.5 years in the study by Waengertner *et al*[24], up to 81.7 mo in the study by Takahashi *et al*[23].

Concerning the MVD assessment method that was applied, the majority of included trials described the use of light microscopy and immunochemistry, implementing the technique proposed by Weidner *et al*[21] (Table 2). Exceptions to this were trials by Imamura *et al*[8] and Waengertner *et al*[24] which reported the application of a modified Horak technique and Chalkley method, respectively. Despite the fact that the majority of eligible trials used CD31 antibodies, Zhao *et al*[25] utilized the CD34 antibody. Heterogeneity was identified in the reported level of magnification. More specifically, the applied magnification spanned from 40X up to 400X. Furthermore, non-uniformity was discovered in the number of spots examined, which ranged from 3 to 10 spots. Only two trials[8,9] confirmed blinded estimation of microvessel density by two independent observers, and none provided information about the existence of separate count for intratumoral and peritumoral vessels. All researchers except Zhao *et al*[25] included the MVD cut-off value in their study articles.

Table 3 summarizes the data regarding the risk classification of included tumours. Moreover, the localization of GISTs included: 9 in the oesophagus, 284 in the stomach, 127 in the small intestine and 28 in the anatomic area of the colon and rectum. According to Table 4, only the study group of Chen *et al* [22] recorded tumor complications like necrosis (37%) and haemorrhage (72.6%). Table 4 incorporates histopathologic characteristics, such as the mitotic count and the tumor size of included GISTs. From the eligible trials, tumor cell type categorization was performed in only 3[8,24,25] studies. In total, 29 epithelioid, 222 spindle and 25 mixed tumours were identified. Finally, inconsistent data were provided by the included trials in terms of the operation performed and chemotherapy type administered.

Risk of bias within studies

Regarding the assessment based on the NOS scale, most studies achieved a 5-star score. The trial by Chen *et al*[22] was an exception, as it appointed a 6 star score. Inter-rater agreement was estimated to be in a very good level (Cohen's k statistic: 86.8%, $P < 0.001$)

Primary endpoint

Data regarding the HR of DFS were extracted from 4 studies (Figure 2). Meta-analysis of these data showed a statistically significant ($P = 0.009$) hazard ratio of DFS (HR: 8.52, 95%CI: 1.69-42.84), in favour of the low MVD group. Since heterogeneity was significant (Q test $P < 0.001$, $I^2 = 90\%$), a RE model was applied.

Due to the high heterogeneity level, further statistical investigation was performed. The first step included a sensitivity analysis for the effect of each study separately. The overall heterogeneity level was not affected by any study. Meta-regression (Supplementary Tables) for the variables sample size, age and follow-up duration did not identify any statistically significant factor. Subgroup analysis regarding the number of study centres and the antibody used were identical to the above-mentioned sensitivity analysis. Analysis of studies implementing the Weidner MVD assessment method showed a statistically significant hazard ratio. Similarly, exclusion of the two studies which did not report blinded MVD evaluation did not influence heterogeneity. Further explanatory analyses (Supplementary Tables) included meta-regression of the primary outcome with the number of spots examined, the percentage of high-risk tumours, gastric and small intestine tumours, large size tumours (≥ 5 cm) and spindle cell

Table 1 Study characteristics, *n* (%)

Ref.	Type of study	Country	Centre	Sample (patients)	Analysis sample	Specimens	Age	Gender (male/female)	Follow-up
Chen *et al*[22], 2005	Retrospective	Taiwan	Single centre	62	59 (3 cases lost to follow-up)	62	24 (38.7) ≤ 61 yr; 38 (61.3) > 61 yr	34 (54.8)/28 (45.2)	50.5 (31) mo for 59 cases
Imamura *et al* [8], 2007	Retrospective	Japan	Single centre	95	95 (80 from the K-M curves)	95	64 (11.667) yr	48 (50.5)/47 (49.5)	48.4 (26.1833) mo for 80 cases
Takahashi *et al* [23], 2003	Retrospective	Japan	Single centre	53	53	53	59.5 (13.3) yr	32 (60.3)/21 (39.6)	81.7 (63.2) mo
Waengertner *et al*[24], 2011	Retrospective	Brazil	Single centre	79	79	79	58.9 (13) yr	42 (53.2)/37 (46.8)	2.5 (2.8) yr
Wang *et al*[9], 2009	Retrospective	China	Multicentre	68	68	68	56.8 (14.75) yr	38 (55.9)/30 (44.1)	42.9 (14) mo for 64 patients
Zhao *et al*[25], 2012	Retrospective	China	Single centre	124	124	124	54.6 (11.667) yr	64 (51.6)/60 (48.4)	52 (32.333) mo

Table 2 Microvessel density assessment

Ref.	MVD assessment method	Antibody	Magnification used	Spots examined	Blinded reading	Observers	Separate count for intra/peritumoral vessels	MVD cut off
Chen *et al*[22]	Light microscopy, immuno-histochemistry	CD31	10X; 20X; 100X	3	N/A	N/A	N/A	15/HPF
Imamura *et al* [8]	Light microscopy, immuno-histochemistry, slight modification of Horak *et al* technique	CD31	40X; 200X	10	Yes	2	N/A	7/0.95 mm²
Takahashi *et al*[23]	Light microscopy, immuno-histochemistry	CD31	40X; 100X; 400X	3	N/A	N/A	N/A	19/HPF
Waengertner *et al*[24]	Light microscopy, immuno-histochemistry, modified Chalkley method	CD31	200X	3 to 5	N/A	N/A	N/A	6 vessels
Wang *et al*[9]	Light microscopy, immuno-histochemistry	CD31	200X	4	Yes	2	N/A	10.54/200HPF
Zhao *et al*[25]	Light microscopy, immuno-histochemistry, Weidner technique	CD34	100X; 200X	5	N/A	N/A	N/A	N/A

MVD: Microvessel density; N/A: Not applicable.

malignancies. A significant correlation was not confirmed with any of the previously mentioned variables.

Secondary endpoints

In total, 3 studies provided data concerning the comparison between high and low MVD groups for DFS at 12 mo (Figure 3). Meta-analysis of these data showed no statistically significant difference (P = 0.13) of DFS (OR: 1.91, 95%CI: 0.83-4.41) at 12 mo between the two study groups. However, a statistically significant difference (P < 0.001) of DFS (OR: 8.46, 95%CI: 3.54-20.19) in favour of the low MVD group was estimated at 36 mo. Although there was no difference (P = 0.58) of DFS rates (Figure 4) at 60 mo (OR: 2.31, 95%CI: 0.12-44.82), the low MVD group displayed a higher (P = 0.0003) DFS rate (Figure 3) at 120 mo (OR: 22.71, 95%CI: 4.11-125.57).

Finally, two studies provided data concerning the development of metastases (Figure 3). Meta-analysis of these data showed a statistically significant (P = 0.0003) lower ratio of metastases (OR: 0.11, 95%CI: 0.03-0.36) in the low MVD group. Heterogeneity was not significant in this analysis (Q test P = 0.29, I^2 =10).

Table 3 Tumor classification, *n* (%)

Ref.	Risk				Location				
	Very low risk	Low risk	Intermediate risk	High risk	Stomach	Small intestine	Colon	Rectum	Esophagus
Chen *et al*[22]	0 (0)	31 (50)	0 (0)	31 (50)	41 (66)	18 (29)	3 (4.8)	0 (0)	0 (0)
Imamura *et al*[8]	7 (7.3)	22 (23.2)	38 (40)	28 (29.5)	64 (67.4)	31 (32.6)	0 (0)	0 (0)	0 (0)
Takahashi *et al*[23]	16 (30.1)		10 (18.8)	27(50.9)	53 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Waengertner *et al*[24]	12 (15.4)	11 (13.8)	18 (23.1)	38 (47.7)	36 (45.6)	30 (38)	0 (0)	0 (0)	0 (0)
Wang *et al*[9]	0 (0)	20 (29.4)	0 (0)	48 (70.6)	28 (41.2)	20 (29.4)	11 (16.2)	0 (0)	0 (0)
Zhao *et al*[25]	6 (4.8)	20 (16.1)	37 (29.8)	61 (49.3)	62 (50)	28 (22.6)	14 (11.3)		9 (7.3)

Risk of bias across studies

Visual inspection of the funnel plot suggested that studies by Wang *et al*[8] and Waengertner *et al*[23] lie beyond the 95%CI limits. Based on Egger's test, there was no statistically significant publication bias (P = 0.517). Exclusion of the above-mentioned trials resulted in a statistically significant HR (7.71 95%CI: 4.02-14.8, P < 0.001) in favour of the low MVD group, though with a limited degree of heterogeneity (Q test P = 0.64, I^2 = 0%).

DISCUSSION

Since GISTs are the most frequently occurring parenchymal neoplasms of the GI tract, research is focused on improving prognosis, introducing novel chemotherapeutic agents and refining current surgical approaches[11-15,33]. Since a few decades ago conventional chemotherapy and radiotherapy did not yield satisfactory results, a R0 resection of the tumor was considered the only therapeutic option for adequate long-term survival[33]. The discovery of the c-kit proto-oncogene mutation and ligand independent activation of the KIT receptor tyrosine kinase in GISTs, resulted in subsequent development of the tyrosine kinase inhibitors imatinib and sunitinib. This led to the onset of targeted molecular therapy of these neoplasms[11,13,33]. In a cohort study by Guller *et al*[34], the Surveillance, Epidemiology and End Results database was screened, with 5,138 GIST patients included. Data analysis revealed that recent advancements in treatment resulted in a significant increase in survival rates of both metastatic (3-year OS: 54.7%, cancer-specific survival: 61.9%) and non-metastatic disease (3-year OS: 88.6%, cancer-specific survival: 92.2%)[34].

It must be noted that despite the above-mentioned novelties, the mortality rate – particularly for the metastatic group - remains high. As a result, various risk grading tools have been developed to quantify the risk and provide accurate prognosis regarding survival endpoints. The study group of Fletcher *et al*[16] proposed the use of primary tumor size and mitotic count as grading parameters, which classified GISTs in four successive categories based on risk of aggressive behaviour. Due to a discrepancy in the metastatic risk between gastric and intestinal GISTs of different grading scores, the primary tumor location was also incorporated[17]. Exporting data from the SSG XVIII trial and using the Z9001 study as a validation tool, Joensuu *et al*[18] suggested that high tumor mitotic count, non-gastric location, large size and tumor rupture were significantly and independently related to a suboptimal recurrence-free survival (RFS).

Besides these grading tools, various independent tumor histopathological factors have been studied for their prognostic value. Specifically, GISTs with an epithelioid or mixed cell type have been associated with a significantly lower 5-year recurrence free survival, when compared with the respective spindle cell tumours (23% *vs* 49%)[35]. Moreover, according to Martin *et al*[36], high tumor cellularity was characterized as a significant poor RFS prognostic factor. Overexpression of Ki67, a nuclear marker abundant in proliferating cells, was found to have an increased incidence in the high risk group[19]. On the contrary, expression levels of p53 in GISTs were not significantly associated with clinical outcomes[37,38]. A pooled analysis from Luo *et al*[20] showed that an elevated neutrophil to lymphocyte ratio was associated with decreased DFS/RFS (HR: 2.18, 95%CI: 1.30-3.67). Furthermore, blood vessel invasion in the primary tumor was suggested as a predictor of liver metastasis and an aggressive behaviour[39].

Angiogenesis in GISTs is considered of the utmost importance for the neoplasm growth and metastasis process[8]. Proliferation of tumor vasculature is achieved *via* the paracrine release of angiogenic molecules and growth factors from tumor and stromal cells[8]. In a recent study by Zhao *et al*[25], the altered expression and secretion of proliferating and angiogenic agents like PI3K, Akt, PTEN, MMP9 and VEGF were directly associated with the DFS in GIST patients. Regarding VEGF, higher serum VEGF values were found in GIST patients when compared to healthy controls, while a positive

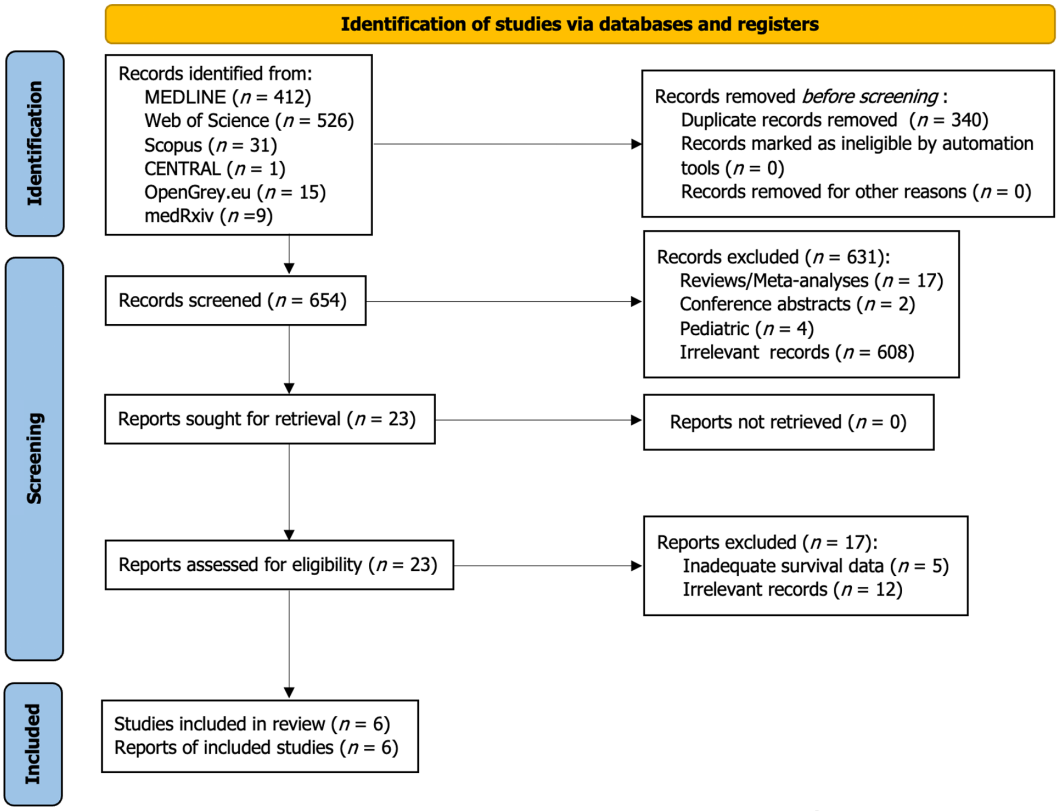
Table 4 Tumor and treatment characteristics, *n* (%)

Ref.	Necrosis		Hemorrhage		Mitotic count		Tumor size		Pcna index		Cell type			Treatment			
	Yes	No	Yes	No					≤ 10%	> 10%	Epithelioid	Spindle	Mixed	Surgery	Surgery type	Chemotherapy	Chemotherapy type
Chen *et al*[22]	23 (37)	39 (63)	45 (72.6)	17 (27.4)	36 (58) < 2/10 HPF	26 (42) ≥ 2/10 HPF	32 (51.6) < 5 cm	30 (48.4) ≥ 5 cm	32 (51.6)	30 (48.4)	N/A	N/A	N/A	Yes	Subtotal gastrectomy, complete tumor resection or segmental enterectomy	Yes (some of them)	See comments
Imamura *et al*[8]	N/A	N/A	N/A	N/A	55 (57.9) < 5/50 HPF	40 (42.1) ≥ 5/50 HPF	39 (41.05) < 5 cm	56 (58.95) ≥ 5 cm	N/A	N/A	1 (1.05)	92 (96.85)	2 (2.1)	Yes	Resection with negative margins	N/A	N/A
Takahashi *et al*[23]	N/A	N/A	N/A	N/A	33 (62.2) < 3/50 HPF	20 (37.7) ≥ 3/50 HPF	21 (39.6) ≤ 3 cm	32 (60.3) > 3 cm	N/A	N/A	N/A	N/A	N/A	Yes	Surgical resection	N/A	N/A
Waengertner *et al*[24]	N/A	N/A	N/A	N/A	N/A	N/A	N/A, varies from 0.5 to 25 cm (median 4.8 cm)		N/A	N/A	N/A	57 (72.2%)	N/A	N/A	N/A	Yes	Adjuvant therapy with tyrosine kinase inhibitors (400mg/daily) for no longer than 3 months
Wang *et al*[9]	N/A	N/A	N/A	N/A	45 (66.2) < 2/10 HPF	23 (33.8) ≥ 2/10 HPF	24 (35.3) ≤ 5 cm	44 (64.7) > 5 cm	N/A	N/A	N/A	N/A	N/A	Yes	N/A	No	No
Zhao *et al*[25]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	28 (22.58)	73 (58.87)	23 (18.55)	Yes	Only biopsy, palliative resection, radical resection	Yes	Postoperative

N/A: Not applicable.

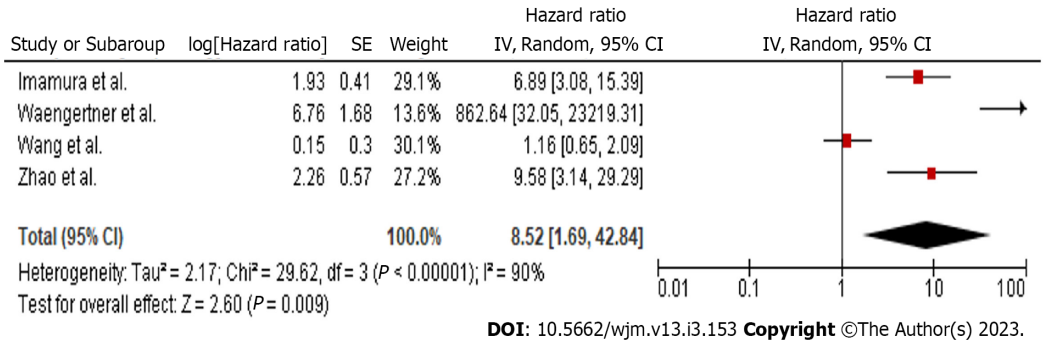
VEGF expression rate was found in high risk groups[9]. A considerable number of clinical trials have correlated high VEGF levels with poor prognosis[9,23,25,40]. Another angiogenic factor, PDGF, has been related to GIST vasculogenesis at both theoretical and clinical levels[41,42]. As tumor angiogenesis often progresses through a hypoxic drive, researchers have correlated the expression levels of respective markers (*e.g.* HIF-1α) with survival outcomes[22,43]. Finally, vasculogenic mimicry (VM) which is a novel pattern of angiogenesis and defined as the formation of fluid conducting channels by highly invasive and dysregulated tumor cells, has also been studied in GISTs[44,45]. MMP-2 and MMP-9 were found to be contributing factors in VM; a significant association between VM, a high mitotic rate and liver metastases was confirmed[44].

Microvessel density is a direct method of quantifying and assessing intratumoral vasculature, and consequently angiogenesis potential. Due to the above-mentioned correlation between tumor vascularity and clinicopathological endpoints, various trials investigated GIST MVD. According to Imamura *et al*[8] and Waengertner *et al*[24], a statistically significant difference of survival rates in favour of low MVD GISTs was reported. Furthermore, Wang *et al*[9] stated that higher MVD values



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Figure 1 PRISMA flow chart.



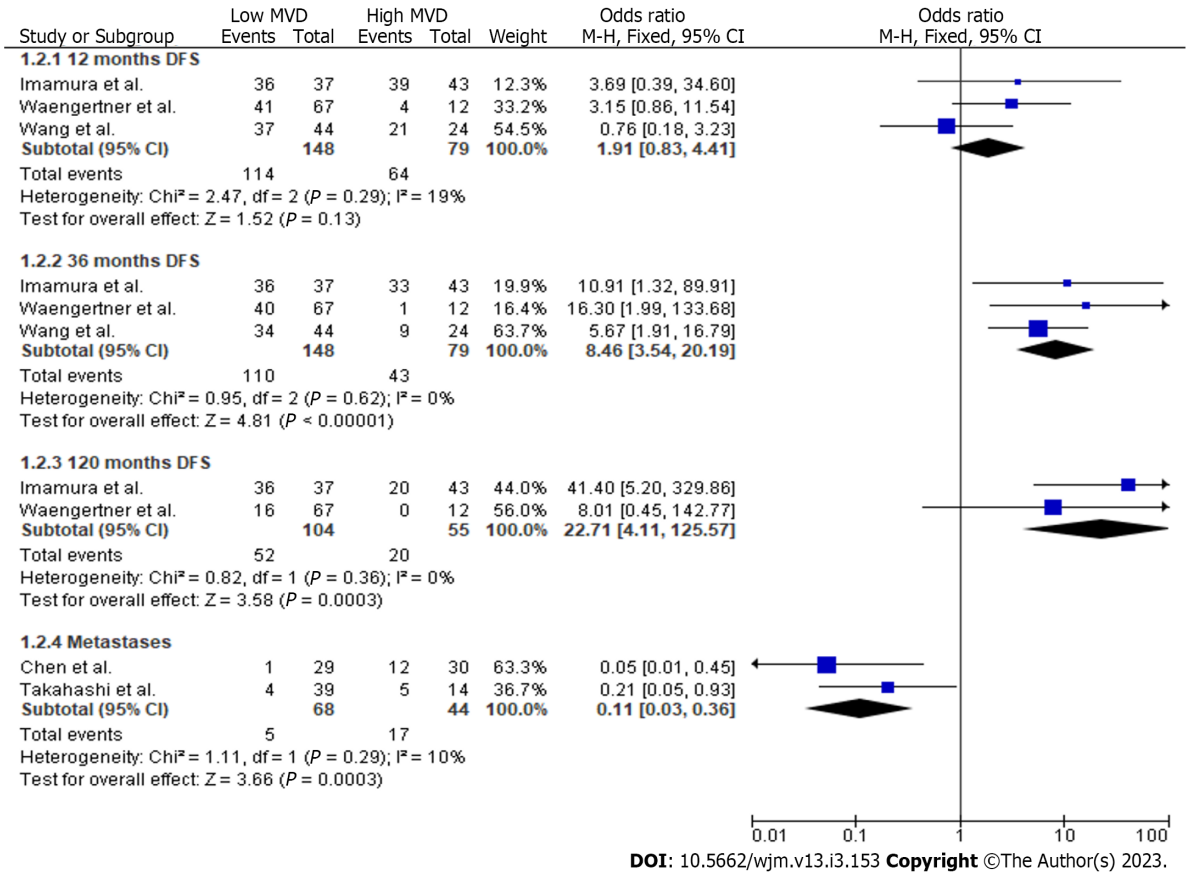
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Figure 2 Hazard ratio of disease free survival.

were found in high mitotic count and recurrence groups. Similar results were published by Zhao *et al* [25], where a significant hazard ratio for DFS was found. A retrospective study by Takahashi *et al*[23] suggested that while high MVD displayed a significant relationship with liver metastases, it did not influence the survival outcome at 10 years.

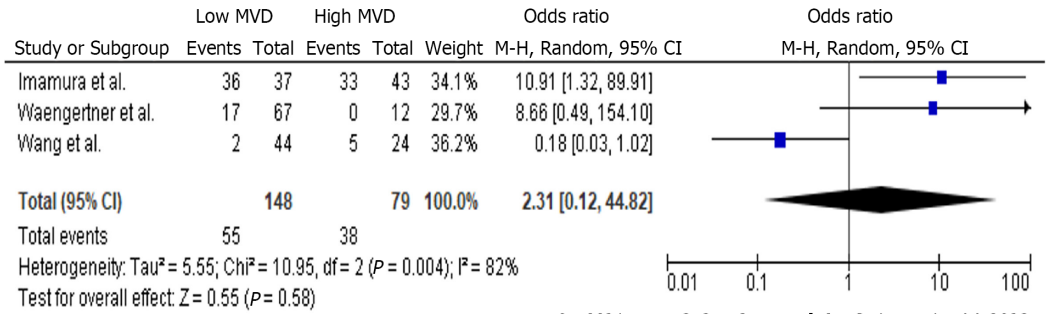
The results of our meta-analysis validated the significance of the MVD value effect on survival. Specifically, higher intratumoral MVD measurements were associated with a lower DFS rate at 36 and 120 mo of follow-up. These were not confirmed at the intermediate endpoints of 12 and 60 mo. The enhanced malignant potential of high vascularized GISTs was also depicted by the significant association among metastatic rate and MVD values.

The usefulness of these results involve extensive approaches in the clinical outcome prognosis[8-9,23, 25,43]. Consolino *et al*[46] showed that in dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), imatinib-resistant tumors had an increased vessel density and permeability, with these attributes significantly correlated with MVD and MDD, respectively. Contrast enhanced endoscopic ultrasound has also demonstrated the ability to assess GIST vascularity, and subsequently, malignant potential[47]. Furthermore, since MVD is a direct tumor vasculature marker, it has been used as an indicator of the angiogenesis inhibition, as well as the overall response to novel medical treatment[48].



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Figure 3 Odds ratios of disease free survival and metastases.



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Figure 4 Sixty months disease free survival.

Besides GIST, MVD assessment has been extensively researched as a means of solid tumor vasculature quantification. Researchers have attempted to identify and estimate the presence of a correlation between microvessel density and survival outcomes in malignancies of the prostate[49], cervix[50], ovaries[51], breast[52], pancreas[53], kidney[54] and lung[55]. Moreover, in two recent meta-analyses from our study group concerning cutaneous melanoma and patients with differentiated thyroid cancer, high intratumoral MVD was related to poor survival outcomes[56,57]. According to current literature, the majority of studies validate the presence of a significant correlation between intratumoral MVD and prognosis in solid tumors[58]. However, a discrepancy exists since a minority of publications question the significance of the above-mentioned correlation[58].

Heterogeneity of various clinicopathological endpoints (survival, metastasis, local recurrence, response to treatment, *etc*.) in the reported results has been widely attributed to certain methodological variations[58]. Among these, selection of the hot-spot examination technique is the most important, due to the variability rates and dependence on the assessor training and experience[59,60]. Furthermore, the MVD assessment technique includes various modifications, such as Weidner's hot-spot method[21], the lumen method[61], Chalkley's method[62] and the computerized image analysis system[63]. Another field of methodological diversity is considered the selection of the endothelial marker, where a variety

of choices such as pan-endothelial cell markers (CD31, CD34, vWF) and selective for the activated endothelium factors (CD105) are described. Finally, technical discrepancies are also reported in other methodological fields, such as type of fixative, vasculature estimation, the MVD cut-off value, level of magnification and overall field size[58]. Our study highlighted this heterogeneity; the use of different assessment methods and definitions of high and low MVD tumours prohibited the calculation of a pooled cut-off point.

Certain limitations should be taken into consideration, prior to appraising results of the present meta-analysis. Firstly, significant levels of heterogeneity were identified; despite conducting explanatory analyses, the validity of study conclusions may be compromised. Furthermore, all eligible studies were designed using a retrospective methodology and included a small sample size, thus allowing the introduction of bias. Moreover, diversity among included studies regarding methodological characteristics of the MVD assessment technique should be also acknowledged. The implementation of different assessment methods and different cut-off points prohibited the strict definition of high and low MVD GISTs. Furthermore, heterogeneity in terms of tumor location, risk classification, histopathological characteristics and cell subtype jeopardized the significance of our outcomes. Inconsistency in surgical or medical treatment could also be an influencing factor on survival endpoints. Finally, since in most trials the raw survival data had to be extracted and reconstructed from the provided Kaplan-Meier curves, a certain amount of bias was introduced, although this procedure has been extensively described and applied in the literature.

CONCLUSION

To the best of our knowledge, the present study is the first attempt to provide an overall estimation of the impact of MVD on survival rates of GIST patients. According to the pooled results of the meta-analysis, GIST allocation between high and low MVD values significantly influenced the DFS hazard ratio. Moreover, high MVD GISTs demonstrated a statistically significant lower DFS at 36 and 120 mo of follow-up, while no difference was found at 12 and 60 mo. Moreover, high MVD tumours were associated with a significantly higher rate of metastases. Based on the above-mentioned results and given several limitations, further studies with a larger sample size and adequate methodology are required.

ARTICLE HIGHLIGHTS

Research background

Several clinical and histopathologic factors have been investigated as prognostic indicators of survival in patients with gastrointestinal stromal tumours (GISTs).

Research motivation

Microvessel density (MVD) has been extensively applied as a direct method of tumour vascularity assessment.

Research objectives

This meta-analysis attempted to estimate the pooled effect of tumoral vascularity based on MVD assessment on the survival of patients with GISTs.

Research methods

The present meta-analysis adhered to the Systematic reviews and Meta-Analyses guidelines and the Cochrane Handbook for Systematic Reviews of Interventions.

Research results

Low vascularized tumours were associated with improved pooled disease-free survival. GISTs with lower MVD values displayed a reduced risk of metastases.

Research conclusions

MVD is significantly associated with the survival outcomes of GIST patients.

Research perspectives

Further prospective randomized controlled trials are required to delineate the exact correlation between MVD and prognosis outcomes in GIST patients.

FOOTNOTES

Author contributions: Perivoliotis K, Ntellas P, Baloyiannis I contributed to study conception and design; Dadouli K, Koutoukoglou P contributed to acquisition of data; Perivoliotis K, Ntellas P, Samara A contributed to analysis and interpretation of data; Perivoliotis K, Ntellas P contributed to drafting of manuscript; Ioannou M, Tepetes K contributed to critical revision.

Conflict-of-interest statement: The authors declare no potential conflicts of interest.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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Country/Territory of origin: Greece

ORCID number: Konstantinos Perivoliotis 0000-0002-6622-5734; Ioannis Baloyiannis 0000-0002-6050-3000; Athina A Samara 0000-0002-6177-7281; Prodromos Koutoukoglou 0000-0002-7083-0718; Panagiotis Ntellas 0000-0003-1708-3861; Katerina Dadouli 0000-0002-8821-0795; Maria Ioannou 0000-0003-2154-9310; Konstantinos Tepetes 0000-0001-9435-6849.

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ABOUT COVER

Peer Reviewer of *World Journal of Methodology*, Ahmad Ozair, MBBS, Postdoctoral Fellow, Miami Cancer Institute, Baptist Health South Florida, Miami, FL 33176, United States. ahmad.ozair@baptisthealth.net

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EDITORIAL

Importance of methodological considerations in documenting psychological trauma

Gentian Vyshka, Fatime Elezi, Tedi Mana

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Gentian Vyshka, Biomedical and Experimental Department, Faculty of Medicine, University of Medicine, Tirana 1005, Albania

Fatime Elezi, Tedi Mana, Service of Psychiatry, University Hospital Center "Mother Teresa", Tirana 1005, Albania

Corresponding author: Gentian Vyshka, MD, Professor, Biomedical and Experimental Department, Faculty of Medicine, University of Medicine, Rr. Dibres 371, Tirana 1005, Albania. gvyshka@gmail.com

Abstract

The documentation of psychological trauma is obviously a challenge to clinicians while they are diving deep into remote events related to their clients or patients. The potential role of psychological trauma in the early developmental stages, and even the existence of adverse childhood experiences, is important to prove, yet it is difficult to do so. A diverse range of methods have been applied, all of which presumably benchmark a big therapeutic step; however, these enthusiastic methods frequently do not last for long. While hypnosis supporters, Freudian and Neo-Freudian disciples can be acute enough to enhance and uncover suppressed memories, modern psychiatry relies mostly on diversely structured interviews. Functional magnetic resonance and its related subtleties might help, but the questions that remain unanswered are numerous and confusing. Connecting early experiences with long-term memory while identifying psychological trauma its importance for the individual's growth trajectory; thus, it remains an intriguing issue.

Key Words: Psychological trauma; Adverse childhood experiences; Post-traumatic stress disorder; Self-reporting; Hypnosis; Magnetic resonance imaging

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Core Tip: The documentation of psychological trauma is a delicate issue with important clinical, ethical and legal implications. Interviews, self-reporting, hypnosis and recent sophisticated imaging techniques have been proposed and tried. While each method has intrinsic advantages and drawbacks, it is important for scholars to specify their chosen approach while denoting the value and limitations of the findings.

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INTRODUCTION

Remote psychotrauma, especially in the early stages of human development, deserves special attention with regard to later psychiatric morbidities. This issue has long since been of particular value as even ancient authors highlighted the role of stress and trauma in the aftermath of catastrophic life events.

Of interest might be that early sources grant no time delay between psychotrauma and its effects on the human psyche. However, months or possibly years may be required for the full picture of posttraumatic stress disorder to arise; this might be true for Homer's Odysseus, who experiences flashbacks and intrusive memories of lost friends while in captivity on Calypso's island, although the legendary author sketches no clear timelines. This notion of time and relativity is older than Einstein's discovery.

Macbeth demonstrated the full picture of psychotrauma soon after the killing of his rival[1]. Charles IX experienced hallucinations and nightmares the day after the Saint Bartholomew massacre: […] the solitary hours of Charles IX were rendered terrible by a repetition of the cries and groans which assailed his ears during the massacre of Saint Bartholomew [2].

Therefore, it is clear that in remote historical settings, the timeline of psychotrauma following a stressful event is different from what it seems to be in current lines of thought. The immediacy of reaction(s) is disputable but remains present.

Self-reporting and structured interviews

The form of trauma reporting in a structured interview differs from the self-reporting found in most available questionnaires; however, the authors generally speak through their characters as if the experiences were their own. Trauma reporting recently transcended the necessity of a close relationship between the physician (therapist) and the patient (client), as questionnaires can be completed online as well. Thus, the effects of psychotrauma might be communicated *via* the internet[3].

It is clear, however, that patient interviews and self-reports have limitations. Memories of remote and early traumatic experiences are often suppressed or erased. Furthermore, identifying participants whose responses are likely dishonest or unreliable is challenging[3]. Documentation requires objectivity; hence, the value of an interview remains restricted. Even when equipment and staffing are adequate, different issues (for example, retraumatization) might lead to the loss of information[4].

Hypnosis and psychoanalysis

The advent of hypnosis and psychoanalytical methodologies provided new perspectives for obtaining the requested information. In 1889, Oppenheim elaborated on some of Erichsen's ideas and coined the term 'traumatic neurosis'. Another French author (Brissaud) pushed the notion further and spoke of 'sinistrosis'[5]. While Erichsen (1866) proposed an organic nature of traumatic events, the London surgeon Page argued in 1885 that it was not physical injuries but rather fright, fear, and alarm that caused the disorder he termed "nervous shock"[6,7]. These views and those of other contemporaries prompted Charcot's pupil, Sigmund Freud, and his apostles to dig deeper into the traumatic unconscious[8,9].

The milestone Freudian publications, namely, 'Interpretation of dreams' (1900) and 'Beyond the pleasure principle' (1920), shed unprecedented light on the internalization of psychological trauma and its elaboration, although not everyone—perhaps even less his contemporaries—shared Freud's opinions[10,11].

Criticism aside, hypnosis was developed before the nineteenth century, yet French scholars maintain its worth; Janet referred to hypnosis as 'influence somnambulique'[12]. Nevertheless, apart from the intrinsic problems of hypnosis, hypnotherapy and all other subtleties of the technique somehow failed to stand the test of time. The changed status of awareness in hypnosis leading to profound relaxation and free production of recollections of remote traumas (if present) does not necessarily serve as documentation of trauma. Mnestic lacunae, confabulations and inconsistent statements arise, even with an experienced therapist. To add more to the controversy, Dreikurs suggested, "Hypnosis will not continue as a therapeutic procedure, despite its present tremendous appeal and increasingly wide use"[13].

Functional neuroimaging and biochemistry

Neuroimaging and neurochemistry have made significant progress in the last half-century. This progress has had clear implications in psychiatry, clinical psychology and psychotrauma.

Table 1 Documenting psychological trauma

Methodology	Advantages	Disadvantages
Self-reporting, structured interviews and questionnaires	Online surveys possible	Interviewer dependent
	User friendly	Participant motivation
	Therapeutic alliance	Low response rate
	Culturally shaped	Risk of retraumatization
	Standardizing of results	
	Quantifiable	
Hypnosis and psychodynamic/psychoanalytical approach	Recall of suppressed memories	Mnestic lacunae
	Recollections of remote traumas and of adverse childhood experiences	Confabulations
	Person centered	Inconsistent statements
	Potentially of treatment value	Potential for false memories
	Exploring the subconscious	Unsuitable for psychotic patients
MRI (functional neuro-imaging)	Objective data/findings	Methodology still not well standardized
	Reproducible	Ethnic and age-related changes to be considered
	Images availability and storage of high quality	Relatively costly

MRI: Magnetic resonance imaging.

Structural magnetic resonance imaging studies on traumatized children and adolescents have revealed abnormalities in numerous brain regions[14]. An impressive number of structures seem involved or imputed. The amygdala has been the focus, with measurements of its volume; the same is true for the hippocampus, brain cortex and limbic system as a whole[15,16]. Most studies have focused on children and childhood trauma, therefore encompassing an age range when important developmental changes occur. This bias is unneglectable but is not the only factor involved. Individual, racial and ethnic changes in volumetric parameters could also alter imaging findings. Researchers have strived to draft reliable and accurate methodologies; however, dilemmas remain.

Psychotrauma has an important effect on the brain's neurotransmitter systems. Therefore, a search is warranted for biochemical markers of trauma, in spite of their delicate and dynamic equilibrium. The hypothalamic pituitary adrenal axis is clearly relevant in stress responses; additionally, several neurotransmitters appear to be involved, such as serotonin and amino acids[17]. Interestingly, some biological therapies have tried to prevent post-traumatic stress disorder symptoms; an example is administering intranasal oxytocin, which is a hypothalamic hormone with a wide range of neural effects[18].

Table 1 summarizes some of the novelties and drawbacks intrinsic to most of the abovementioned methodologies. In a nonexhaustive approach, authors have tried to focus on the main characteristics of each technique, which obviously have numerous and specific features.

CONCLUSION

The theme of psychological trauma and its long-term after-effects is of particular interest in neuroscience. However, this issue still needs systematization. Documenting a psychologically remote and traumatic event might be problematic but of high value when medico-legal and forensic issues are at stake, even with the unfortunate result of retraumatization[19].

When producing results, clinicians must clearly report the methodology used for documenting psychological trauma. By doing so, everyone should clearly understand the limitations of the findings without raising unnecessary doubts about their validity.

FOOTNOTES

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Country/Territory of origin: Albania

ORCID number: Gentian Vyshka 0000-0001-5286-1265; Tedi Mana 0000-0002-3723-675X.

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OPINION REVIEW

ChatGPT in action: Harnessing artificial intelligence potential and addressing ethical challenges in medicine, education, and scientific research

Madhan Jeyaraman, Swaminathan Ramasubramanian, Sangeetha Balaji, Naveen Jeyaraman, Arulkumar Nallakumarasamy, Shilpa Sharma

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Madhan Jeyaraman, Naveen Jeyaraman, Arulkumar Nallakumarasamy, Department of Orthopaedics, ACS Medical College and Hospital, Dr MGR Educational and Research Institute, Chennai 600077, Tamil Nadu, India

Swaminathan Ramasubramanian, Sangeetha Balaji, Department of General Medicine, Government Medical College, Omandurar Government Estate, Chennai 600018, Tamil Nadu, India

Shilpa Sharma, Department of Paediatric Surgery, All India Institute of Medical Sciences, Delhi 110029, New Delhi, India

Corresponding author: Madhan Jeyaraman, MS, PhD, Assistant Professor, Research Associate, Department of Orthopaedics, ACS Medical College and Hospital, Dr MGR Educational and Research Institute, Dr MGR Educational and Research Institute, Chennai 600077, Tamil Nadu, India. madhanjeyaraman@gmail.com

Abstract

Artificial intelligence (AI) tools, like OpenAI's Chat Generative Pre-trained Transformer (ChatGPT), hold considerable potential in healthcare, academia, and diverse industries. Evidence demonstrates its capability at a medical student level in standardized tests, suggesting utility in medical education, radiology reporting, genetics research, data optimization, and drafting repetitive texts such as discharge summaries. Nevertheless, these tools should augment, not supplant, human expertise. Despite promising applications, ChatGPT confronts limitations, including critical thinking tasks and generating false references, necessitating stringent cross-verification. Ensuing concerns, such as potential misuse, bias, blind trust, and privacy, underscore the need for transparency, accountability, and clear policies. Evaluations of AI-generated content and preservation of academic integrity are critical. With responsible use, AI can significantly improve healthcare, academia, and industry without compromising integrity and research quality. For effective and ethical AI deployment, collaboration amongst AI developers, researchers, educators, and policymakers is vital. The development of domain-specific tools, guidelines, regulations, and the facilitation of public dialogue must underpin these endeavors to responsibly harness AI's potential.

Key Words: Artificial intelligence; ChatGPT; Open artificial intelligence; Education; Research

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Core Tip: Chat Generative Pre-trained Transformer's potential applications span medicine, education, and scientific research, with a focus on addressing its limitations and ethical concerns. Emphasizes the importance of developing guidelines and fostering collaboration between artificial intelligence (AI) developers, tech companies, researchers, and academic publishers. The key areas for future advancements, such as enhancing AI capabilities, addressing biases, and promoting the ethical use of AI tools have been outlined.

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INTRODUCTION

"Chat Generative Pre-trained Transformer (ChatGPT) is an advanced artificial intelligence (AI) language model developed by OpenAI, based on the Generative Pre-trained Transformer (GPT)-4 architecture. Stemming from the original GPT model inspired by the 2017 Transformer architecture, successive iterations culminated in ChatGPT, which generates human-like text and performs tasks like text completion, summarization, and translation. Despite its remarkable capabilities, ChatGPT faces limitations such as sensitivity to input phrasing, plausible yet incorrect responses, and inconsistent world knowledge. Ongoing research aims to enhance the model's performance and address these challenges". That is an exemplary introduction to the topic of this paper, offered by ChatGPT when asked to explain its nature and historical development within a 100-word limit. It serves as an apt teaser to the scope of capabilities of the system. Within a week of its release, ChatGPT reached one million users, reflecting its wide acceptance and potential impact[1-4]. The model has evolved from GPT-1 through GPT-3, with the latter using 45 TB of data to enhance its ability to generate coherent sequences of words, code, and data. The latest iteration, GPT-4, was released on 14 March 2023. However, it still faces limitations such as the inability to consistently answer semantic, factual, and ethical questions, as well as the presence of biases and prejudices[5-8]. Despite these drawbacks, early reactions to ChatGPT have been largely positive, with users acknowledging its transformative potential across various domains. This review article provides an overview of the current state of ChatGPT, discussing its applications, challenges, and potential impact. Additionally, we will explore possible measures to incorporate ChatGPT into the healthcare and scientific realms, ensuring its utility is maximized while mitigating risks.

AI AND HUMAN INTELLIGENCE: COMPLEMENTARY FORCES SHAPING THE FUTURE OF SCIENCE AND EDUCATION

AI technology, particularly large language models (LLMs) such as ChatGPT, has demonstrated remarkable capabilities in generating human-like text, answering questions, and providing explanations[9,10]. These advancements have significantly impacted various fields, including medicine[11-13], law[14,15], and academia[9,16-18]. ChatGPT has the potential to revolutionize science by speeding up the article writing and editing process, allowing researchers to focus on their research[19-22]. In education, AI chatbots like ChatGPT can offer students individualized learning experiences, assistance in learning new languages, tutoring and homework help, and answers to challenging questions[23].

AI systems, such as ChatGPT, have shown potential in various fields, including medical education, clinical decision support, and genetics[9,12,13,15,16]. For instance, ChatGPT has been evaluated in the context of the American Heart Association Basic Life Support and Advanced Cardiovascular Life Support exams, performing reasonably well overall, with accuracy varying depending on the question type[15,24]. In another study, ChatGPT was compared to human respondents in addressing genetics questions, showing similar performance but excelling in memorization-style questions[12,13]. Additionally, ChatGPT performed well on standardized exams such as AMBOSS-Step1, AMBOSS-Step2, NBME-Free-Step1, and NBME-Free-Step2 datasets[12,13].

However, it is essential to acknowledge the significance of human involvement in scientific research, as humans are responsible for posing hypotheses, designing experiments, and interpreting results[25]. Thorp[25] emphasizes the role of machines as tools but highlights that the scientific record ultimately results from human endeavors grappling with critical questions. Furthermore, OpenAI and DeepMind have developed AI systems, ChatGPT and AlphaCode, capable of producing lines of code. Although these systems have the potential to automate certain tasks in large software engineering projects, understanding human needs can be challenging, as these needs may be difficult to describe with

machine-readable specifications[11].

Limitations and potential misuse of AI raise concerns about their impact on human intelligence[23,26,27]. Neural network-based LLMs may pose a challenge for scientific thinking, as they are trained on past information and may struggle to think differently from the past, potentially hindering social and scientific progress[28]. Additionally, biases and inaccuracies may arise from the quality of training datasets[23]. Irene Solaiman, a researcher of the social impact of AI at Hugging Face, has expressed concerns about relying on these models for scientific thinking[28].

As AI systems continue to evolve, it is crucial to strike a balance between AI advancements and human intervention. AI technology can enhance various aspects of human life, but the significance of human involvement in tasks such as posing hypotheses, designing experiments, and interpreting results should not be overlooked[25]. AI systems can be viewed as tools that complement and extend human intelligence rather than replacing it. While AI has the potential to augment human intelligence, it is unlikely to completely take over, as human creativity, critical thinking, and adaptability remain indispensable in various aspects of life and scientific research.

CHATGPT IN HEALTHCARE

ChatGPT has demonstrated significant potential in various healthcare-related applications, such as medical education, radiologic decision-making, clinical genetics, and patient care[9,12-16]. Its use in medical education has shown promise as an interactive tool to support learning and problem-solving[9]. ChatGPT performed at or near the passing threshold for all three United States Medical Licensing Exam (USMLE) exams and demonstrated a high level of concordance and insight in its explanations without any specialized training or reinforcement[13].

ChatGPT has potential applications in healthcare education, research, and practice, such as improving scientific writing, enhancing research equity and versatility, streamlining workflow, saving time, and improving health literacy[16,21,29]. In the context of nursing education, O'Connor[23] suggests using a variety of assessment methods to reduce the risk of automated answers in students' written work, while emphasizing the importance of educating students about academic integrity and the value of critical thinking and scientific writing. AI chatbots like ChatGPT have the potential to be valuable tools for tailored learning experiences in education, providing personalized learning experiences for students, assisting with language acquisition, providing homework help and tutoring, and answering questions to aid in understanding complex concepts[23]. A publication discusses how doctors can use ChatGPT to write medical summaries for patients, emphasizing the importance of considering patient acceptance of new technology and its potential negative effects[17]. However, concerns have been raised about the lack of transparency and accountability in AI-generated content[30].

Research by Rao *et al*[12] suggests that specialized AI-based clinical decision-making tools will emerge in the future, highlighting the potential of using ChatGPT for radiologic decision-making and finding it feasible and potentially beneficial for improving clinical workflow and responsible use of radiology services. Another paper by Kung *et al*[13] concludes that LLMs, such as ChatGPT, have the potential to enhance the delivery of individualized, compassionate, and scalable healthcare by assisting with medical education and potentially clinical decision-making. In the field of clinical genetics, Duong and Solomon[15] found that ChatGPT did not significantly differ from humans overall in answering genetics questions, but performed better on memorization-type questions than critical thinking questions. The study also revealed that ChatGPT frequently provided different answers when asked the same question multiple times, with plausible explanations for both correct and incorrect answers[15]. Fijačko *et al*[24] tested ChatGPT's accuracy in answering questions related to life support and resuscitation, revealing that it could provide accurate answers to a majority of the questions on the American Heart Association's Basic Life Support and Advanced Cardiovascular Life Support exams. Furthermore, ChatGPT may assist with individualized, compassionate, and scalable healthcare delivery[13].

In neurosurgical research and patient care, ChatGPT has been explored for its potential role in gathering patient data, administering surveys or questionnaires, and providing information about care and treatment[14]. However, the implementation of these technologies should be approached carefully to ensure effectiveness and safety[14]. The potential of combining biotechnology and AI to tackle global issues and advance sustainable development goals is examined in a paper, which covers the wide range of AI applications in the life sciences, including decision support, natural language processing, data mining, and machine learning[31]. The authors emphasize the value of reproducibility in the development of AI models and highlight current research issues and challenges in these fields[31]. Another article explores the use of computational systems biology in stem cell research, emphasizing the value of computational methods in understanding the intricate biological mechanisms underlying stem cell differentiation and regeneration[32]. The article also highlights the importance of interdisciplinary partnerships between computational and experimental biologists to advance stem cell research. Furthermore, it discusses the application of machine learning and deep learning algorithms in stem cell research, serving as an example of how computational systems biology can advance our knowledge of stem cells and their potential therapeutic uses[32].

AI-powered chatbots, like ChatGPT, have the potential to improve patient outcomes by facilitating communication between patients and healthcare professionals, informing patients about their care and treatment using natural language processing[14]. In the context of neurosurgical research, chatbots can speed up the process of data collection and analysis by gathering information from a large number of patients and may be useful for longitudinal studies, which must track patient outcomes over time[14]. In a study that investigates various ChatGPT prompting strategies for breast cancer screening and breast pain, it was suggested that ChatGPT can be used to help radiologists choose the best imaging modalities, potentially enhancing clinical workflow and encouraging the prudent use of radiology services[12]. An article covering the third year of the coronavirus disease-19 pandemic, along with various topics like computer-based testing

(CBT), study design, ChatGPT, journal metrics, and appreciation for reviewers, emphasizes the benefits of CBT and suggests adding more multimedia test items to better represent real-world clinical scenarios[33]. The significance of the study design and the application of ChatGPT for data analysis are also discussed in the article[33].

ChatGPT has demonstrated significant potential in various healthcare-related applications, ranging from medical education to clinical decision-making and patient care. Its implementation in these fields has yielded notable results, but it is essential to approach the integration of such technologies carefully to ensure effectiveness, safety, and ethical considerations.

NAVIGATING THE ETHICAL AND QUALITY CHALLENGES OF AI-GENERATED CONTENT IN MEDICAL EDUCATION AND RESEARCH

The use of AI models such as ChatGPT in medical education, decision-making, scientific writing, and research has demonstrated their capabilities and potential benefits[9,12,13,15,21,22,29]. However, their utilization comes with potential risks and challenges, including ethical, copyright, transparency, legal issues, and concerns related to the generation of content difficult to distinguish from human-generated content[16,26]. Moreover, there are issues of bias, plagiarism, lack of originality, inaccurate content, and incorrect citations[16,34]. Several articles emphasize the importance of transparency, integrity, and truth in scientific research and highlight the potential risks associated with the use of AI tools like ChatGPT[20,26]. To address these concerns, the development of appropriate guidelines, regulations, and technologies for detecting AI-generated outputs and ensuring the safe and responsible use of ChatGPT and other LLMs in healthcare, academia, and research has been proposed[16,26,27,34]. The academic community must address these challenges by investing in the development of robust quality control measures for AI-generated content, including stringent human supervision, validation of generated content, and ensuring that AI-generated outputs meet high standards of accuracy, originality, and integrity[29,34]. In addition, funders, publishers, and research institutions should adopt clear policies to encourage openness and public understanding of the use of AI-generated content[20].

Several key issues need to be discussed, including the potential for AI-generated content to be used unethically, the need for transparency and honesty, the risk of manipulating public opinion or decision-making, the necessity of policies and guidelines, and the potential for AI-generated content to transform research practices and publication[20]. Furthermore, academic publishers should engage in discussions about the implications of AI-generated content and create comprehensive guidelines for publishing such content[30]. Notably, according to recent articles, it can be difficult for journal editors to recognize and reject papers written by AI because these models can often produce texts that look very similar to those written by humans[22]. The use of GPT-3 as a co-author in a study published in the journal Oncoscience has sparked a debate about authorship conventions in the context of AI-generated content[35]. While some experts argue that AI shouldn't be given credit for writing, others highlight their role in generating ideas and producing papers. This ongoing discussion may lead to changes in authorship conventions in the future[35].

The rise of AI models like ChatGPT presents both opportunities and challenges in the fields of medical education, scientific writing, and research. Addressing the ethical, legal, and quality concerns associated with AI-generated content requires the collective efforts of the academic community, publishers, and AI developers. By proactively establishing guidelines, regulations, and policies, the potential benefits of AI-generated content can be realized while minimizing the risks associated with its misuse.

BALANCING THE BENEFITS AND ETHICAL IMPLICATIONS OF CHATGPT IN HEALTHCARE AND EDUCATION

ChatGPT, a powerful AI tool with potential applications across various fields, has been the subject of numerous studies and discussions. Despite its promise in reducing the time required for tasks, it has several limitations and ethical implications that need to be considered, particularly in sensitive fields like healthcare and education[11,20,22,28-30,36]. One primary concern is the accuracy of the information generated by ChatGPT, as it heavily depends on the quality of its training data[23,37]. The risk of biased or misleading results due to poor-quality datasets is particularly relevant in fields like medical education and clinical decision-making, which require high levels of precision[12,13,24]. Such inaccuracies may negatively impact patient outcomes and damage the reputation of the medical community, while in the realm of education, they could lead students astray and impede learning[25,26,29,34]. O'Connor[23] warns against relying on automated tools like ChatGPT to detect plagiarism in nursing education, suggesting a combination of assessment methods, including oral presentations and objective structured clinical examinations, along with smaller pieces of scientific writing. This approach would reduce the risk of automated answers in students' work and emphasize the importance of academic integrity, critical thinking, and scientific writing skills. Additionally, other papers discourage the use of ChatGPT or similar technologies for writing research articles or cheating on assignments[10,26]. Another limitation of ChatGPT is its inability to perform well on tasks requiring critical thinking or reasoning[15,36]. Duong and Solomon [15] acknowledges that the model's performance may not generalize to all types of genetics questions or contexts, as it performs better on memorization-type questions than on critical thinking ones. This indicates that relying solely on AI models like ChatGPT may not be sufficient for addressing complex tasks in various fields, including healthcare and education. Moreover, ChatGPT is prone to generating fake references and citations, a phenomenon referred to as "hallucination" or "stochastic parroting"[22]. This poses a significant challenge for journal editors, as the output may

contain fabricated information, undermining the credibility of scientific research. The potential misuse of ChatGPT for plagiarism also raises concerns[10,16,23,26,36], which could disrupt traditional methods of assigning essays and lead to a decline in academic integrity.

In addition to the aforementioned concerns, the utilization of ChatGPT poses several other challenges, including the dangers of blind trust, excessive regulation, dehumanization, misaligned optimization targets, information overload, false forecasting, and the need for self-reference monitoring[38-40]. A study found that there are worries about the possible dangers of using GPT-3 for online radicalization and extremism. The model can be manipulated to amplify extremist ideologies, generate polemics reminiscent of past attackers, and propagate harmful beliefs. Efforts should be made to understand and mitigate these risks effectively[41]. Another study emphasizes the need to handle privacy and Personally Identifiable Information retention issues when employing AI-powered chatbots, especially in delicate situations like summarising cover letters. It underlines the requirement for responsible AI use and the creation of privacy protection measures[42]. Moreover, language models like ChatGPT can give rise to various detrimental outcomes, such as discrimination, toxicity, information hazards, misinformation propagation, malicious exploitation, issues with human-computer interaction, and environmental impacts[39,40]. These challenges underscore the significance of responsible usage and achieving a balance between regulation and innovation.

The integration of AI-generated content raises questions about authenticity, accountability, privacy, and security[1,18]. As a result, there is an urgent need for guidelines and regulations to ensure the safe and responsible use of ChatGPT and other LLMs. Chatterjee and Dethlefs[27] emphasizes the responsibility of tech companies like OpenAI to provide solutions to manage potential misuse and ensure the ethical use of powerful AI models. The Lancet article also calls for investment in detecting problematic outputs and establishing editorial policies that keep up with evolving technology to ensure the safe and ethical use of AI tools like ChatGPT in scholarly publishing[34]. The reliance on LLMs like ChatGPT for scientific thinking may impede social and scientific progress, as these models are trained on past information and may not be able to think differently from the past[28]. Researchers and academics should remain vigilant in their use of AI tools, emphasizing human involvement in hypothesis formulation, experimental design, and interpretation of results to ensure that the scientific record remains a product of human endeavor in grappling with critical questions[25].

Other key ethical issues brought up by the use of ChatGPT include those bias, authorship, privacy and security, transparency, abuse, and privacy and security. One major concern is the potential for bias in ChatGPT's responses, stemming from biases present in the training data, model bias, and non-representative data labelers. This bias can perpetuate and amplify societal biases, leading to unfair and discriminatory outcomes. Privacy and security are also significant issues, as ChatGPT collects data during training, including potentially sensitive personal information, and user interactions with the system may inadvertently disclose personal details, posing risks if obtained by malicious entities. Another ethical concern is the lack of transparency in ChatGPT's decision-making process and the limited disclosure of technical details by OpenAI, making it challenging for users to have control over the generated content and understand the model's limitations. Furthermore, there are concerns regarding the potential for abuse, including the spread of misinformation and the impersonation of individuals using ChatGPT[38,40].

A review of the primary literature on AI-assisted psychosis risk screening in adolescents focuses on two specific methods: chatbot-based screening and analysis of large-scale social media data. The authors highlight ethical issues as the primary challenge in utilizing AI for psychiatric risk screening. They emphasize the need for compliance with the biomedical ethical principles of respect for autonomy, non-maleficence, beneficence, and impartiality[43]. A different study on ChatGPT's moral authority shows that it has the potential to enhance users' moral judgement, but also emphasizes its inconsistency and capability to taint judgement. Users underestimate its power, making responsible use and instruction in digital literacy necessary to effectively understand its recommendations[44]. Another paper, on the other hand, offers proof that language models developed using reinforcement learning from human feedback (RLHF) are capable of "moral self-correction" by avoiding damaging outputs when specifically told to do so. This ability, which involves the models' capacity for comprehension and instruction following, arises with larger models trained with RLHF. The results point to the possibility of teaching language models to follow moral standards, providing cause for cautious hope[45]. A review of the primary literature on AI-assisted psychosis risk screening in adolescents focuses on two specific methods: Chatbot-based screening and analysis of large-scale social media data. The authors highlight ethical issues as the primary challenge in utilizing AI for psychiatric risk screening. They emphasize the need for compliance with the biomedical ethical principles of respect for autonomy, non-maleficence, beneficence, and impartiality.

While ChatGPT offers numerous benefits, its limitations and ethical implications must be carefully considered. Publishers, funders, and researchers must establish clear rules and regulations regarding the use of ChatGPT and similar tools in academic research[11,20,22,28-30,36,38-40]. For instance, in healthcare, AI-generated summaries using patient data should be manually reviewed by a healthcare professional before use, taking into account patient acceptability and potential consequences of technology failure[17]. Transparency and accountability should be prioritized by researchers and developers by disclosing data on bias, privacy, and system constraints. Vulnerable users should be given extra protection, and it's crucial to communicate clearly about ChatGPT's capabilities and restrictions. ChatGPT responses should be justified and given only in response to specific requests. The accuracy of ChatGPT's responses can be improved by including domain-specific knowledge through expert-curated data. At the same time, users must validate information, tell facts from fiction, comprehend ramifications, communicate clearly, and be aware of terms and conditions. Regulators and politicians should strive for a balanced strategy that steers clear of overregulation and prevents information and communication concentration. To handle complex ethical issues, ethicists need to work in conjunction with specialists from a variety of professions[38]. It is advised, in terms of regulation policy, to put measures in place that stop the abuse of ChatGPT in academic settings, such as using different exam formats and applying AI content detectors for plagiarism detection. The issue of including ChatGPT as a co-author in academic works should be addressed with clear norms and disclosure requirements. It's important to note that ChatGPT-generated content isn't protected by copyright[40]. The

development of risk assessment frameworks and tools to evaluate the potential risks connected to language models should be an area of focus of future studies. It is necessary to incorporate disciplines like ethnographic research and human-computer interaction into the methodological toolbox for language model analysis. Furthermore, to create efficient plans for resolving identified hazards, technological and sociotechnical mitigation research is required. To establish normative performance levels, benchmarking initiatives should be conducted with input from all participants. To fully understand the possible advantages and overall social impact of language models, a thorough investigation should be done[39]. Overall, a cautious and well-considered approach is necessary for the integration of AI tools like ChatGPT, with an emphasis on maintaining human involvement in critical aspects of research, assessment, and decision-making processes. By doing so, we can ensure the responsible and ethical use of AI while leveraging its benefits in various fields.

THE FUTURE OF AI AND HUMAN INTELLIGENCE - INTEGRATING CHATGPT RESPONSIBLY

The ChatGPT has demonstrated remarkable potential in various fields, including medicine, education, law, and scientific research[9,11,15,16,18]. This large language model exhibits proficiency in answering questions, producing well-referenced writing, assisting in drafting papers, and providing support for clinical decision-making[9,12,13,15,21]. However, it is important to recognize the limitations and potential risks of AI, as human intelligence, creativity, and critical thinking remain essential components of scientific inquiry and progress. AI tools should be viewed as complementary rather than a replacement for human expertise. In medical education and clinical decision-making, ChatGPT has been found to perform at or near the passing threshold for USMLE[13], highlighting its potential as an interactive medical education tool and in assisting with radiologic decision-making, streamlining clinical workflow, and improving responsible use of radiology services[12]. Nevertheless, its application in these fields should be approached with care, acknowledging its limitations, the risk of biased or misleading results, and the importance of human involvement in the decision-making process[23,25,29].

In scientific publishing, ChatGPT has demonstrated the capacity to accelerate the writing and editing process[21]. However, concerns about the authenticity and accountability of AI-generated content have been raised[30,34]. Researchers suggest that academic publishers engage in discussions about the implications of AI-generated content and establish comprehensive guidelines for publishing such content[30]. Moreover, some studies emphasize the need for collaboration in preventing potential misuse of AI models like ChatGPT and call for tech companies to take responsibility for managing potential misuse[27]. An article published in Nature highlights the issues that require attention, such as the tasks that should be delegated to LLMs, the qualifications and skills necessary for researchers, the stages of the AI-assisted research process that need human verification, and the laws required to deal with LLMs[20]. There should also be discussions on how LLMs can be used to educate and train researchers, how researchers and funders can encourage the development of independent, open-source LLMs, and what standards of quality should be expected of LLMs. Further topics include how LLMs can advance open science principles and research equity, as well as the legal ramifications LLMs may have on scientific practice[20].

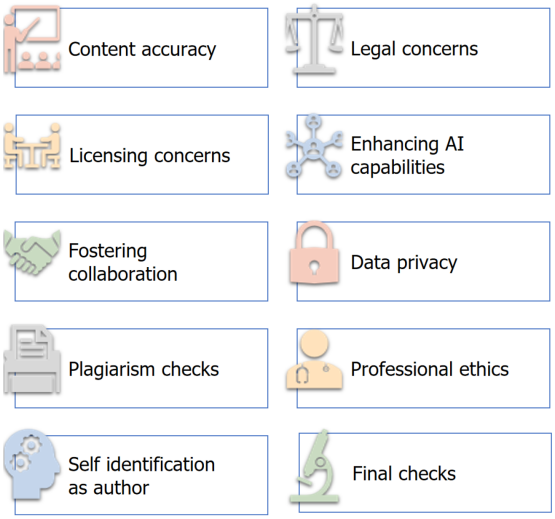
The development of ChatGPT's ability to interact with humans naturally and ask follow-up questions to reduce bias holds great promise for its future applications[27]. By using reinforcement learning to optimize the model, ChatGPT becomes more robust and capable of sustaining longer conversations with users. The potential of ChatGPT extends beyond the examples mentioned in the paper, including prospects for its use in various industries such as customer service, education, and mental health[27].

As AI technologies continue to advance, they have the potential to revolutionize numerous fields, including healthcare, education, and scientific research. However, there are several key considerations for future developments, as highlighted by the cited research: (1) Enhancing AI capabilities: Future AI models should aim to improve their performance on critical thinking tasks, as well as memorization-type questions, to be more effective in assisting human experts in various fields [15]; (2) Addressing biases: AI developers should work to minimize biases in AI systems, as these biases can limit the effectiveness and ethical use of AI tools[25]; (3) Ensuring ethical use: Tech companies and researchers must prioritize the ethical use of AI tools and develop strategies to prevent misuse[27]. This includes investing in methods to detect problematic outputs and establishing editorial policies that can adapt to evolving technology[34]; and (4) Fostering collaboration: The integration of AI and human expertise should be encouraged, promoting a collaborative approach that leverages the strengths of both AI and human intelligence[10]. The challenges of ChatGPT is depicted in Figure 1.

By focusing on these key areas, future advancements in AI can help address current limitations and ensure that the technology is employed responsibly and ethically. Through this approach, AI can be harnessed to improve healthcare, education, and scientific research, all while maintaining the importance of human intelligence and critical thinking.

CONCLUSION

ChatGPT has shown significant potential in revolutionizing various fields, including science, healthcare, and education, by accelerating processes, enhancing personalization, and providing valuable support to professionals and learners alike. Despite its capabilities, it is important to recognize that ChatGPT is not a substitute for human intelligence and its use comes with an array of ethical, legal, and quality-related challenges that need to be addressed to harness its full potential. Establishing clear guidelines and usage policies is essential to ensure the responsible integration of ChatGPT in academic and professional settings. This includes maintaining transparency in AI-generated content, acknowledging the potential



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Figure 1 Challenges of ChatGPT.

for misinformation and plagiarism, and promoting adherence to quality standards. Furthermore, as AI systems like ChatGPT continue to advance, continuous research, interdisciplinary collaboration, and dialogue among stakeholders are crucial in addressing the limitations, risks, and ethical implications of this emerging technology.

In the healthcare sector, for instance, striking a balance between the benefits of AI assistance and the potential risks associated with misinformation is of utmost importance. Close monitoring, human verification, and careful consideration of patient acceptability are necessary to mitigate these risks. Similarly, in education, it is essential to maintain academic integrity and discourage any unethical use of ChatGPT while exploring its potential for personalized learning experiences. As the landscape of AI-assisted research and applications continues to evolve, it is important to examine the broader implications of ChatGPT and similar tools on the job market, legal frameworks, and societal values. The development of open-source LLMs, the establishment of standards of quality, and the evaluation of legal ramifications are topics that warrant further exploration.

While ChatGPT holds great promise in transforming various industries and enhancing research and learning experiences, it is essential to adopt a cautious and responsible approach to its integration. By fostering interdisciplinary collaboration, ongoing research, and proactive policy development, the research community can ensure that conversational AI is utilized ethically, effectively, and responsibly, paving the way for innovative applications that positively impact society.

FOOTNOTES

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Country/Territory of origin: India

ORCID number: Madhan Jeyaraman 0000-0002-9045-9493; Swaminathan Ramasubramanian 0000-0001-8845-8427; Sangeetha Balaji 0000-0002-1566-1333; Naveen Jeyaraman 0000-0002-4362-3326; Arulkumar Nallakumarasamy 0000-0002-2445-2883; Shilpa Sharma 0000-0001-8695-8372.

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REVIEW

Compensated liver cirrhosis: Natural course and disease-modifying strategies

Ramesh Kumar, Sudhir Kumar, Sabbu Surya Prakash

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Ramesh Kumar, Sudhir Kumar, Sabbu Surya Prakash, Department of Gastroenterology, All India Institute of Medical Sciences, Patna 801507, India

Corresponding author: Ramesh Kumar, MD, Additional Professor, Department of Gastroenterology, All India Institute of Medical Sciences, 4th floor, IPD Block-C, Patna 801507, India. docrameshkr@gmail.com

Abstract

Compensated liver cirrhosis (CLC) is defined as cirrhosis with one or more decompensating events, such as ascites, variceal haemorrhage, or hepatic encephalopathy. Patients with CLC are largely asymptomatic with preserved hepatic function. The transition from CLC to decompensated cirrhosis occurs as a result of a complex interaction between multiple predisposing and precipitating factors. The first decompensation event in CLC patients is considered a significant turning point in the progression of cirrhosis, as it signals a drastic decline in median survival rates from 10-12 years to only 1-2 years. Furthermore, early cirrhosis has the potential to regress as liver fibrosis is a dynamic condition. With the advent of effective non-invasive tools for detecting hepatic fibrosis, more and more patients with CLC are currently being recognised. This offers clinicians a unique opportunity to properly manage such patients in order to achieve cirrhosis regression or, at the very least, prevent its progression. There are numerous emerging approaches for preventing or delaying decompensation in CLC patients. A growing body of evidence indicates that treating the underlying cause can lead to cirrhosis regression, and the use of non-selective beta-blockers can prevent decompensation by lowering portal hypertension. Additionally, address-ing various cofactors (such as obesity, diabetes, dyslipidaemia, and alcoholism) and precipitating factors (such as infection, viral hepatitis, and hepatotoxic drugs) that have a detrimental impact on the natural course of cirrhosis may benefit patients with CLC. However, high-quality data must be generated through well-designed and adequately powered randomised clinical trials to validate these disease-modifying techniques for CLC patients. This article discussed the natural history of CLC, risk factors for its progression, and therapeutic approaches that could alter the trajectory of CLC evolution and improve outcomes.

Key Words: Compensated cirrhosis; Compensated advanced chronic liver disease; Clinical decompensation; Cirrhosis reversal; Disease-modifying agents; Acute-on-chronic liver failure

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Core Tip: Compensated liver cirrhosis might be reversible if the underlying cause is treated before the disease progresses. The median survival for these individuals is typically 10-12 years; however, after the first decompensation, it drastically drops to 1-2 years. As a result, the outcomes of such patients can be significantly improved by integrating a number of disease-modifying therapy strategies that address complex pathophysiology, risk factors, and triggering events linked with disease progression. This article discussed the natural course of compensated liver cirrhosis, risk factors for its progression, and potential therapeutic strategies to favourably influence its natural evolution and enhance outcomes.

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INTRODUCTION

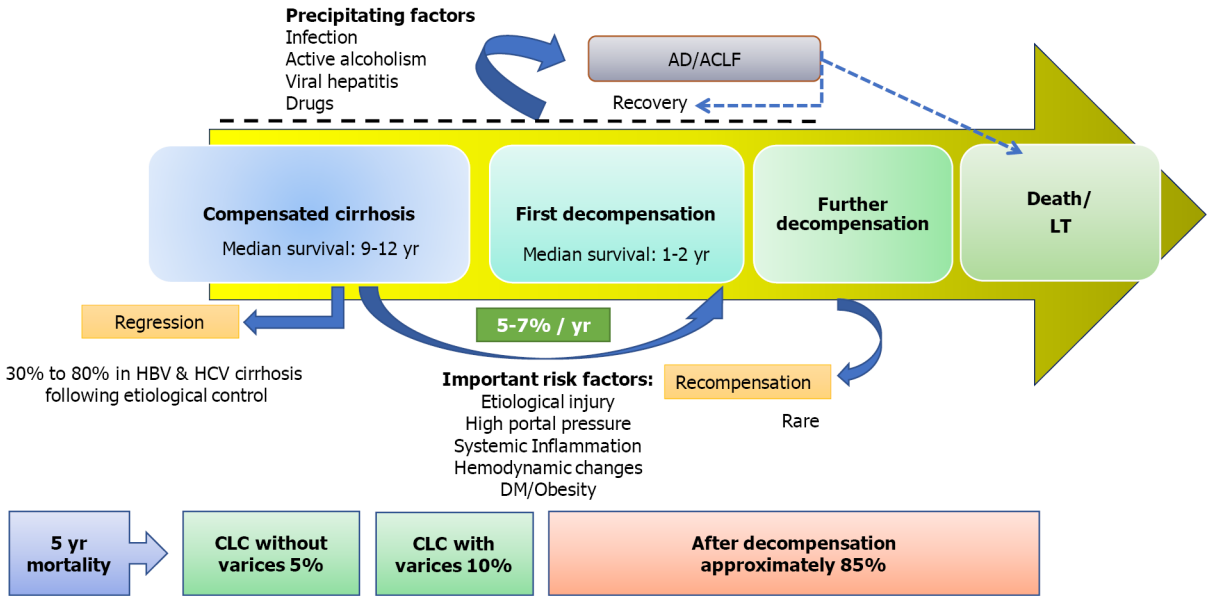
The prevalence and mortality associated with liver cirrhosis (LC) continue to increase despite improvements in knowledge and medical care. According to data from the United States, the annual number of LC-related deaths has risen by 65%, while the number of hospitalisations for LC has nearly doubled in a decade[1,2]. LC has traditionally been regarded as a singular entity with a continuum of increasing degrees of severity until death or liver transplantation. Recently, these paradigms have shifted, leading to the recognition of LC as a heterogenous condition with varying prognosis across the different stages[3,4]. The term "compensated liver cirrhosis" (CLC) is used to describe LC without one or more decompensating events, such as ascites, hepatic encephalopathy, variceal haemorrhage (VH), and jaundice [5]. After experiencing a decompensation event, LC patients are always classified as decompensated LC (DLC) because the pathogenic mechanisms that caused the decompensation persist. When LC patients were separated into two groups based on decompensating events, the median 1-year survival in CLC patients was 95% compared to 61% in DLC patients [6]. Therefore, the first decompensation in CLC patients is regarded as a prognostic watershed due to a substantial reduction in median survival from 10-12 years in CLC to only 1-2 years in DLC[6].

LC has long been viewed as the end stage of chronic liver disease (CLD). However, this perception has started to shift in the past two decades. Wanless *et al*[7] were the first to describe the reversal of LC, and since then numerous series of LC patients with diverse aetiologies have demonstrated the same[7,8]. Patients with CLC remain asymptomatic and undiagnosed for the first few years[9]. Despite being asymptomatic, between one-third and one-half of CLC patients have varices and clinically significant portal hypertension (CSPH) at the time of diagnosis[10-12]. Over time, CLC patients develop several risk factors that increase their susceptibility to clinical decompensation, such as rising portal pressure, systemic inflammation, and haemodynamic changes. Moreover, certain triggers including bacterial infection, medications, or alcohol can acutely precipitate decompensation. When the underlying cause of CLC is eliminated early on, a significant proportion of patients experience cirrhosis regression[7,8].

Even when regression of LC is not possible, there are variety of evolving strategies for preventing or delaying decompensation in such patients. Therefore, the prognosis of such patients can be greatly enhanced by early diagnosis of CLC. However, the medical community has predominantly focused its efforts on managing and improving the outcomes for patients with DLC, with little attention given to the medical management of CLC. In order to enhance the ease of diagnosis of advanced CLD noninvasively using transient elastography, the Baveno VI consensus introduced the new term "compensated advanced CLD" that encompasses CLC and CLD with advanced fibrosis[13]. Due to efficient noninvasive testing tools, more and more LC patients are now being recognised at an early compensated stage[14]. This offers the gastroenterologists and hepatologists greater opportunities to intervene and alter the trajectory of the natural evolution of CLC. This article discussed the natural history of CLC, risk factors for its progression and decompensation, and potential therapeutic strategies to change the course of the illness and improve the outcomes.

NATURAL HISTORY OF CLC

The natural progression of LC is characterised by a continuum from a long silent compensated phase to a more progressive symptomatic decompensation phase (Figure 1). As LC progresses over time, patients develop a variety of risk factors, including altered liver architecture, portal hypertension (PHT), systemic inflammation, and haemodynamic alterations that increase the risk for clinical decompensation. Decompensation may occur insidiously due to slowly increasing portal pressure and deteriorating hepatic function, often referred to as non-acute decompensation (AD)[5]. However, different triggering events, such as bacterial infection, alcohol, bleeding, medications, or a flare-up of liver disease, can lead to AD within days, which may progress to acute-on-chronic liver failure (ACLF). In patients with CLC, decompensation represents a turning point in terms of mortality risk, patient quality of life, and propensity for hospitalisation.



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Figure 1 Natural course of patients with compensated liver cirrhosis. The natural progression of cirrhosis is characterised by a continuum from an asymptomatic compensated phase to a symptomatic decompensation phase. The rate of transition from a compensated to a decompensated stage is about 5%-7% each year. The 5-year mortality rate in compensated cirrhosis without or with varices is 5% and 10%, respectively. There are several known factors associated with decompensation such as high portal pressure, persistent etiological injury, systemic inflammation, and haemodynamic alterations. In addition, several types of triggering events, such as bacterial infection, alcohol, viral hepatitis, or medications, may cause acute decompensation (AD) and acute-on-chronic liver failure (ACLF). Prompt and effective control of etiological factors are associated not only with regression of compensated cirrhosis but also with recompensation of decompensated cirrhosis (data adapted from references 3, 6, 18, and 31). CLC: Compensated liver cirrhosis; DM: Diabetes mellitus; HBV: Hepatitis B virus; HCV: Hepatitis C virus; LT: Liver transplantation.

In a systematic review, pooling of data from relevant studies, revealed that the survival of patients with LC varied from 1 mo to 186 mo, with a median survival > 12 years for CLC and 1.8 years for DLC[6]. The rate of transition from a compensated to a decompensated stage is approximately 5%-7% each year[15]. Ascites is typically the first sign of decompensation in most studies. Overall, the 5-year mortality rate in CLC patients is only 1.5% for those without CSPH, 5.0% for those with CSPH but no varices, and 10.0% for those with CSPH and varices, highlighting the significance of PHT in mortality risk[3]. Therefore, CLC patients without varices and without CSPH constitute a highly compensated group with a very low mortality risk[6].

The first decompensation of CLC does not always indicate a point of no return in the natural course of LC. Emerging data suggests that although it is an uncommon occurrence, recompensation of DLC is possible if the underlying cause of LC is suppressed[16]. Recompensated cirrhosis is indeed a real condition, and the Baveno VII consensus has provided a standard definition for it[17]. Similarly, it is now more widely acknowledged that CLC can regress to a non-cirrhotic stage when etiological factors are promptly controlled[7,18]. Liver fibrosis is a dynamic condition, and early LC, which lacks extracellular matrix crosslinking and marked angiogenesis, can even revert into normal architecture[19].

FACTORS ASSOCIATED WITH DECOMPENSATION OF CLC

The transition from CLC to DLC occurs as a result of a complex interaction between predisposing and precipitating factors (Table 1). The development of PHT is the key factor causing the switch from CLC to DLC[10,20-22]. In a study, patients with a hepatic venous pressure gradient (HVPG) < 10 mmHg had a 90% probability of not developing clinical decompensation over 4 years. As the HVPG rises above 10 mmHg, which signifies CSPH, the risk of decompensation begins to rise[10]. VH typically occurs when HVPG is higher than 12 mmHg[15]. Another study found that CLC with a baseline HVPG > 20 mmHg had a 47% risk of decompensation in mean duration of just 1.6 years[21]. There is also growing evidence that long-term use of non-selective beta-blockers (NSBBs) significantly reduces the risk of decompensation[22]. Thick fibrous septa and small nodules observed in liver biopsy specimens of CLC patients are associated with CSPH and an increased risk of decompensation[23-25]. Ongoing liver damage caused by etiological factors also increases the risk of decompensation. This is supported by the finding that attaining a sustained virological response (SVR) in hepatitis C virus (HCV)-cirrhosis and maintaining viral suppression in hepatitis B virus (HBV)-cirrhosis significantly lowers the incidence of decompensation[26-29]. The neurohormonal and inflammatory alterations in LC contribute to decompensation in the form of ascites by causing splanchnic vasodilatation and lymphatic dysfunction[30-32].

Table 1 Factors associated with decompensation of compensated liver cirrhosis

Risk factors for non-acute decompensation	Precipitating factors for acute decompensation
Thick fibrous septa and micronodularity on liver biopsy	Bacterial infection
Persistent liver injury by etiological factor	Active alcoholism
High portal pressure	Gastrointestinal haemorrhage
Systemic inflammation & hemodynamic changes	Consumption of hepatotoxic drug/alternative medicine
Metabolic risk factors: DM, obesity, and dyslipidaemia	Superinfection or flare of viral hepatitis
Genetic risk factors: *PNPLA3* G/G genotype	Major surgery and general anaesthesia

DM: Diabetes mellitus; *PNPLA3*: Patatin-like phospholipase domain-containing protein 3.

Several metabolic factors have also been found to influence the risk of decompensation[33-36]. CLC patients with diabetes have a higher risk of developing any decompensating event than those without diabetes[33]. Obesity has a negative impact on the natural course of CLC, regardless of aetiology, and increases the risk of decompensation[34]. Sarcopenia and myosteatosis, which are common in CLD with various aetiologies, appear to promote the progression of CLD to advanced stages[35]. Another study found that the *PNPLA3* G/G genotype, involved in triacylglycerol hydrolysis, was associated with a 2-fold increase in the probability of decompensation[36]. Gut dysbiosis, characterised by a loss of beneficial commensals and an increase in pathogenic organisms, significantly influences the natural course of LC patients[36-40]. Furthermore, cirrhosis-associated immune dysfunction, involving both immune deficiency and proinflammatory immune cell activation, contributes to haemodynamic disturbances and PHT, accelerating the development of decompensation[41-43].

Bacterial infection can cause AD by escalating the intensity of systemic inflammation and PHT. In a large prospective study of 1672 patients with compensated HCV-related or HBV-related cirrhosis, bacterial infections preceded and precipitated decompensation in 13% of patients over a 5-year period[44]. Overall, bacterial infection is considered to be the most frequent precipitant of AD (22%-29%) and ACLF (33%-50%)[45]. Alcoholic hepatitis can cause decompensation in patients with CLC through various mechanisms[46,47]. AD and ACLF frequently develop in patients with LC undergoing surgery [48]. In a study using an animal model of CLC, it was discovered that extrahepatic surgery raises the portal pressure during the postoperative period, leading to decompensation[49]. Other situations where decompensation can develop in patients with CLC include superimposed viral hepatitis, consumption of hepatotoxic medications, and vascular thromboses[50].

DISEASE-MODIFYING TREATMENT STRATEGIES

The present strategy for managing patients with LC is centred on strategies intended to avoid or treat complications, without giving much thought to their effects on the natural history of LC. There is a need to pay more attention to agents that target key points in the complex pathophysiology of LC. The ideal goal of a disease-modifying medication should be the regression or reversal of cirrhosis. If this is not possible, the next goal should be to prevent or at least delay the progression of the disease. Growing evidence suggests that addressing the underlying cause of LC and reducing PHT by NSBB have positive impacts on the natural history of patients with CLC[7,8,22,26,29]. Moreover, such patients may potentially benefit from addressing a number of cofactors and precipitating factors that have a negative influence on the natural course of LC[51]. Thus, effective disease-modifying treatment strategies might include: (1) Removal of etiological factors; (2) Pathophysiology-oriented therapy; (3) Management of adverse cofactors like obesity, DM, dyslipidaemia, and alcoholism; (4) Anti-fibrotic and regenerative therapies; and (5) Elimination of precipitating factors that lead to AD/ACLF.

Removal of etiological factors

The main prerequisite for fibrosis regression is the cessation of liver injury, which is accomplished through therapeutic control of causal factors. Regression of LC has been extensively described in patients with HBV-related and HCV-related cirrhosis after aetiological treatment (Table 2). Nevertheless, robust data supporting the regression of non-viral causes of LC are generally lacking.

Viral cirrhosis: Dienstag *et al*[26] reported regression of LC in 73% (8/11) of patients following 3 years of lamivudine therapy. In another prospective study, treatment with adefovir dipivoxil for up to 240 wk resulted in regression of bridging fibrosis or cirrhosis in 58% (7/12) of patients[27]. Pegylated interferon and ribavirin combination therapy has been shown to reduce liver fibrosis following SVR. Combination therapy led to the reversal of LC in 49% of patients in a pooled data analysis from four randomised controlled trials (RCTs) that included 153 patients with HCV-cirrhosis at baseline[52]. Though data are still evolving, a recent study found that direct-acting antivirals were effective in reducing fibrosis based on FibroScan[53]. In a systematic review of 463 patients with HBV-cirrhosis, regression of LC was observed in 33% to 80% of patients following sustained viral suppression. Meanwhile, LC regressed in 33% to 100% of 58 patients

Table 2 Impact of etiological treatment on regression of liver cirrhosis

Ref.	Study design	Drug/duration	Patients, *n*	Baseline LC	Main results
Dienstag *et al*[26], 2003	Prospective, partially randomised	Lamivudin/3 yr	63 CHB	11	LC regressed in 8 of 11 patients (73%)
Hadziyannis *et al*[27], 2006	Prospective	Adefovir dipivoxil, up to 240 wk	125 CHB	4	58% had reversal of bridging fibrosis/cirrhosis; 3 of 4 LC patients had reversal
Marcellin *et al*[29], 2013	Randomised trial	TDF/adefovir for 48 wk then open-label TDF	641 CHB	96	71 of 96 (74%) became non-cirrhotic at 5 yr
Poynard *et al*[52], 2002	Pooled data from RCTs	IFN/PEG-IFN + RBV	3010 CHC	153	The reversal of LC was observed in 75 patients (49%)
Mauro *et al*[53], 2018	Retrospective	DAAs/IFN + RBV	112 HCV-infected LT recipients	37	Regression of fibrosis in 43% of LC (16/37)
Lassailly *et al*[55], 2020	Prospective	Bariatric surgery	180 obese NASH	9	At 5 yr, fibrosis regression was seen in 68% of advanced fibrosis and 33% of patients had reversal of LC
Sanyal *et al*[54], 2022	Data from two RCTs	Simtuzumab or selonsertib or placebo	1135 NASH patients, 709 (62%) had Ishak stage 6 fibrosis	709	LC regression occurred in 16% (176/1135). Drugs were not better than placebo
Dufour *et al*[64], 1997	Retrospective	Immunosuppressant	8 AIH cirrhosis	8	LC regressed in all
Czaja *et al*[63], 2004	Retrospective	Corticosteroid	87 AIH	14	LC regressed in 4 of 14 patients
Bardou-Jacquet *et al*[66], 2020	Retrospective	Venesection	106 patients with haemochromatosis	66	LC regressed in 15 of 66 (23%) during median follow-up of 9.5 yr

AIH: Autoimmune hepatitis; CHB: Chronic hepatitis B; CHC: Chronic hepatitis C; DAAs: Direct-acting antivirals; HCV: Hepatitis C virus; IFN: Interferon; LC: Liver cirrhosis; LT: Liver transplantation; NASH: Nonalcoholic steatohepatitis; PEG-IFN: Pegylated interferon; RBV: Ribavirin; RCT: Randomised controlled trial; TDF: Tenofovir disoproxil fumarate.

with HCV-cirrhosis following SVR[28]. This suggests that once the causal element is removed early in the course of CLC, progression is halted and cirrhosis regression occurs in a significant number of patients.

Non-viral cirrhosis: Weight loss through lifestyle changes improves fibrosis in patients with nonalcoholic steatohepatitis (NASH), but its effects on NASH-cirrhosis per se are still poorly understood. In a recently published study involving 709 patients with compensated NASH-cirrhosis from two RCTs on simtuzumab and selonsertib *vs* placebo, regression of LC was observed in 135 patients during a median follow-up of 16.6 mo. Notably, the impact of the drug was not better than placebo, indicating the influence of lifestyle modification[54]. Another study that assessed the long-term effects of bariatric surgery in 180 obese patients with NASH found significant regression of fibrosis at 5 years after surgery. The fibrosis decreased in 70.2% of patients, disappeared in 42.0% of patients, and 33.0% of patients with baseline LC became non-cirrhotic[55]. However, the outcomes of RCTs on a variety of compounds that target the metabolic/inflammatory pathways of NASH-cirrhosis have been dismal[56-59]. In a recent RCT with emricasan, an oral pan-caspase inhibitor, there was a small treatment effect on HVPG reduction in compensated NASH cirrhosis; however, the drug was overall ineffective in improving clinical outcomes[56]. Belapectin, an inhibitor of galectin-3 that was earlier found to reduce liver fibrosis and PHT in rats, was proven to be ineffective in human NASH-cirrhosis[57].

Abstinence from alcohol improves the prognosis in all stages of alcohol-related LC; nevertheless, there is scant clinical support for fibrosis/cirrhosis regression in alcoholic liver disease[60-62]. In one study, patients who were abstinent *vs* those who were not showed a 3-year decompensation likelihood of 32.4% *vs* 60.0%, respectively[60]. Early alcohol abstinence after LC diagnosis was found to be a significant predictor in survival, with abstinent patients having a 72% survival rate at 7 years compared to 44% in patients who continued to consume alcohol[61]. Reversibility of hepatic fibrosis has also been documented in autoimmune hepatitis patients[63,64]. A study on corticosteroid-treated autoimmune hepatitis has revealed regression of histological LC from 16% to 11%[63]. Ursodeoxycholic acid treatment may halt disease progression and improve the survival of patients with primary biliary cholangitis, but it appears to be less effective in promoting fibrosis regression[65]. In a retrospective analysis of patients with haemochromatosis treated with venesection, LC regression was observed in 15 out of 66 (23%) over a median period of 9.5 years[66].

Pathophysiology-oriented therapy

NSBB: In patients with LC, CSPH appears to be an important pathophysiologic driver of the first decompensating event [10,20-22]. The onset of PHT is primarily caused by elevated portal blood inflow resistance resulting from architectural

distortion (fixed component) and increased elevated hepatic microvascular tone (dynamic component). The increased hepatic vascular tone is attributed to decreased intrahepatic vasodilators, primarily nitric oxide (NO), and increased production of vasoconstrictors such as angiotensin, endothelins, and prostanoids. Portosystemic collaterals appear later, heralding splanchnic vasodilation, increased splanchnic blood flow, and hyperkinetic circulation, all contributing to further raise portal pressure. The only pharmacological class that is still recommended for long-term treatment of PHT is NSBB. Importantly, NSBBs are only beneficial in patients with CSPH and not in those with subclinical PHT because they reduce portal venous flow, and significant hyperdynamic circulation only develops once CSPH is set[67]. Adequate responses to NSBBs are associated with a lower incidence of decompensating events and improved survival rate, indicating a positive influence on the natural history of LC (Table 3).

According to current recommendations, patients with high-risk varices should have NSBBs or endoscopic variceal ligation (EVL) as primary prophylaxis for VH[68]. While choosing between NSBBs and EVL, the patient's preferences, tolerance, side effect profile, and contraindications can all be taken into account[69]. Importantly, NSBB, in addition to being as effective as EVL at preventing VH, has the added benefit of lowering the risk of decompensation and mortality [22,70-73]. Among commonly used NSBBs, propranolol and nadolol reduce portal pressure by reducing portal venous inflow by blocking β_1 and β_2 adrenergic receptors, whereas carvedilol has additional intrinsic vasodilatory activity because of its anti-α-adrenergic activity and its ability to increase NO release[74]. Moreover, carvedilol has been observed to cause greater reduction in HVPG compared to propranolol or nadolol[75,76]. Carvedilol might be especially useful for patients with CLC, where a higher hepatic vascular resistance is the main cause of PH[76,77].

The emerging evidence strongly indicates that NSBBs can prevent decompensation in patients with CLC. The PREDESCI trial, a multicentre, double-blind, RCT, studied whether the administration of NSBBs over an extended period of time prevents the development of clinical decompensation and increase survival in CLC patients with CSPH[22]. In this study, 201 patients with CLC with CSPH were randomly assigned to receive NSBBs or a placebo. After a median follow-up of 37 mo, the NSBB arm showed considerably lower rates of clinical decompensation compared to the placebo arm [17% *vs* 27%, hazard ratio (HR): 0.51, 95% confidence interval (CI): 0.26-0.97]. Among decompensating events, decreased incidence of ascites (20% *vs* 9%) was the main effect of NSBB. However, the PREDESCI trial hinged the choice of NSBB on the HVPG response to intravenous propranolol, which is impracticable for a large population of patients. In a recent competing-risk time-to-event meta-analysis that included 352 patients with CLC, 181 carvedilol-treated patients, and 171 controls from 4 RCTs, long-term carvedilol therapy was associated with a decreased risk of decompensating events and improved survival. Carvedilol decreased the risk of decompensation, mainly ascites, with a subdistribution HR of 0.506 (95%CI: 0.289-0.887), and the risk of death with a subdistribution HR: of 0.417 (95%CI: 0.194-0.896)[78].

Statins: Several studies have been published in recent years on the beneficial effects of statins in patients with LC. The benefits include a decrease in portal pressure, favourable effects on sinusoidal endothelial function, hepatic microcirculation, and liver fibrosis[79]. In the first such study of statins in LC, simvastatin was found to increase NO generation in liver sinusoids and reduced intrahepatic vascular resistance[80]. Furthermore, it was found that simvastatin had an additive effect with NSBB on HVPG reduction[81]. Several moderate-quality studies have suggested that the use of statins in CLC lowers the risk of hepatic decompensation and mortality[82-84]. In a large study from Taiwan, including 1350 patients with LC, statin use decreased the risk of decompensation, mortality, and hepatocellular carcinoma in a dose-dependent manner. With statins, the incidence of decompensation was 61% lower in HBV-cirrhosis and 49% lower in HCV-cirrhosis[85].

In a recent systematic review and meta-analysis, statins were associated with a 46% reduced risk of hepatic decompensation and a 46% lower risk of mortality in patients with LC[86]. Notably, LC patients who benefit from statins are mainly those with Child-Pugh classes A or B but not those with Child class C. In DLC, simvastatin at higher doses may even cause rhabdomyolysis and hepatotoxicity[87,88]. Therefore, despite a strong case for statins being helpful for patients with LC, more evidence from high-quality RCTs is required before they can be regularly recommended for patients with CLC. Until then, it is safe to presume that patients with CLC with dyslipidaemia should not be denied statin therapy.

Anticoagulant: As LC progresses, endothelial damage and occlusion of small hepatic veins causes parenchymal extinction, which contributes to tissue collapse and architectural distortion[8]. Furthermore, studies have shown that the oral anticoagulant rivaroxaban and the low molecular weight heparin enoxaparin lower intrahepatic vascular resistance in cirrhotic rat models[89,90]. These beneficial effects of anticoagulants were attributed to a decreased intrahepatic microthrombosis, hepatic stellate cell deactivation, and increased NO bioavailability. In a small RCT, a 12-mo regimen of enoxaparin in patients with LC with a Child-Pugh score of 7 to 10 appeared to prolong survival and delay the emergence of hepatic decompensation[91]. In a recent meta-analysis, use of antiplatelet agents was associated with a 32% decreased risk of hepatic fibrosis[92]. Still, more data are required before a conclusive statement can be made about the usage of anticoagulants and antiplatelet agents in CLC patients.

Management of co-factors

Weight loss and glycaemic control: Obesity is a condition characterised by systemic inflammation and immunological dysregulation that is linked to poor clinical outcomes in LC patients, including decompensation[34,93]. Obese patients with LC have higher levels of inflammatory cytokines, increasing the risk of decompensation through a systemic inflammatory response[94]. Obesity has a negative impact on the course of CLC across all aetiologies, regardless of portal pressure or liver function. In a study including 161 patients with CLC, clinical decompensation occurred in 15% of lean, 31% of overweight, and 43% of obese patients during a median follow-up of 59 mo[34]. Class III obesity was found to be an independent risk factor for the development of ACLF in a recent study using data registries[95]. Hence, weight loss

Table 3 Impact of non-selective beta-blockers on portal hypertension, variceal haemorrhage, decompensation, and survival in patients with liver cirrhosis

Ref.	Study population	Intervention	Study design	Sample size, *n*	Study conclusion
Poynard *et al* [70], 1991	LC patients with oesophageal varices	Propranolol, nadolol *vs* placebo	Meta-analysis of 4 RCTs	589	Both propranolol and nadolol were effective in preventing first VH and reducing the mortality associated with VH
Tripathi *et al* [71], 2009	LC patients with grade II or more varices	Carvedilol *vs* EVL	RCT	152	On intention-to-treat analysis, carvedilol had lower rates of the first VH compared to EVL (10% *vs* 23%)
Gluud *et al* [69], 2012	LC patients with high-risk varices without prior VH	NSBBs *vs* EVL	Meta-analysis of 19 RCTs	1504	Both EVL and NSBB reduced VH (RR: 0.69 and 0.67) without difference in mortality rates
Sinagra *et al* [75], 2014	LC patients with PHT	Carvedilol *vs* propranolol	Meta-analysis of 5 studies	175	Carvedilol reduced PHT significantly more than propranolol
Bhardwaj *et al*[76], 2017	LC patients with small varices	Carvedilol *vs* placebo	RCT	140	Carvedilol is safe and effective in delaying the progression of small to large oesophageal varices in LC patients
Zacharias *et al*[77], 2018	Adults with LC and varices	NSBBs	Meta-analysis of 10 RCTs	810	Carvedilol was more effective at reducing the HVPG. However, it was not better than traditional NSBBs with regard to the mortality, VH, or adverse events
Malandris *et al*[72], 2019	LC patients requiring primary or secondary prevention of VH	Carvedilol, NSBBS, EVL	Meta-analysis of 13 RCTs	1598	Carvedilol was as efficacious and safe as standard-of-care interventions for the primary and secondary prevention of VH. Also, carvedilol was associated with lower all-cause mortality compared to EVL
Sharma *et al* [73], 2019	LC patients with large oesophageal varices and no prior history of VH	NSBB, isosorbide-mononitrate, carvedilol, and EVL alone or in combination	Meta-analysis of 32 RCTs	3362	NSBB monotherapy decreased all-cause mortality and the risk of first VH. Additionally, NSBB carried a lower risk of serious complications compared with EVL
Villanueva *et al*[22], 2019	CLC patients and CSPH	Propranolol, carvedilol *vs* placebo	RCT	201	Long-term treatment with β blockers could increase decompensation-free survival in patients with CLC with CSPH, mainly by reducing the incidence of ascites
Villanueva *et al*[78], 2022	LC patients with CSPH	Carvedilol *vs* EVL/no treatment	Meta-analysis of 4 RCTs	352	Long-term carvedilol therapy reduced decompensation and significantly improved survival

CLC: Compensated liver cirrhosis; CSPH: Clinically significant portal hypertension; EVL: Endoscopic variceal ligation; HVPG: Hepatic venous portal gradient; LC: Liver cirrhosis; NSBBs: Non-selective beta-blockers; PHT: Portal hypertension; RCT: Randomised controlled trial; RR: Relative risk; VH: Variceal haemorrhage.

may be an effective therapeutic strategy for obese patients with cirrhosis[96,97]. Following bariatric surgery, the advantages of weight loss have been established in obese patients with LC[96]. Nonetheless, bariatric surgery is not generally recommended for people with CLC and is contraindicated in DLC. Patients with LC may be safely recommended to change their lifestyles under the care of a dietician with the aim to reduce body weight.

In LC patients, the prevalence of abnormal glucose regulation, including type 2 DM and hepatogenous diabetes, is quite high (20% to 70%)[98,99]. This high incidence appears to be due to poor insulin clearance and dysfunctional pancreatic beta cells[98]. Hyperinsulinemia is also implicated in vasodilatory and antinatriuretic effects in patients with CLC[100]. Impaired glycaemic indices in LC are associated with disease progression, decompensation, complications, and poor outcomes[101,102]. Hence, maintaining appropriate glycaemic control can benefit the course of LC. However, standardised diabetes management for LC patients has not been established yet. In addition to lifestyle modification, oral hypoglycaemic agents can be used in LC patients up to Child-Pugh class B, while insulin is recommended for LC at all stages.

Alcohol abstinence: Abstinence from alcohol reduces the risk of decompensation and improves outcomes in all stages of alcohol-related LC[60-62]. Early alcohol abstinence after diagnosis of LC is important for a better outcome[61]. According to a meta-analysis of seven cohort studies involving 1235 patients with alcoholic cirrhosis, at least 1.5 years of abstinence is required before a statistically significant difference in survival between the abstinent and alcohol consumption groups can be observed[103].

Diet, salt, and physical activity: Dietary interventions should include a target protein intake of 1.2-1.5 g/kg/d and regular aerobic exercise to prevent or ameliorate sarcopenia, which is associated with poor outcomes in LC patients[104-106]. In a recent prospective study, 16 wk of personalised hypocaloric normoproteic diet and 60 min per week of supervised physical activity were found to reduce body weight and portal pressure in overweight/obese patients with

CLC[107].

Many observational studies have found an inverse relationship between coffee consumption and LC[108,109]. According to two recent meta-analyses, coffee drinkers had a lower risk of developing LC than non-drinkers[108,109]. Unfortunately, these meta-analyses may not have adequately controlled the bias and confounding factors due to the low quality of the observational studies. Prospective RCTs are needed to establish the impact of coffee consumption on fibrosis regression in CLC patients.

Most of the scientific societies do not recommend sodium (salt) restriction for patients with CLC. However, preascitic LC patients have been found to retain sodium when faced with a high salt intake[110]. It is believed that a new steady state of sodium balance is eventually achieved in such patients primarily because of increased levels of atrial natriuretic peptide, inhibition of the renin-angiotensin-aldosterone system, and suppression of sympathetic activity, which prevent sodium retention and the formation of ascites. Jalan *et al*[111] reported that the severity of PHT contributed to the abnormalities of sodium handling in patients with CLC. Therefore, it would be intriguing to determine whether salt restriction could prevent the development of ascites in CLC patients with CSPH.

Correction of vitamin D deficiency: The liver plays a crucial role in 25-hydroxyvitamin D metabolism. Vitamin D deficiency and insufficiency are highly prevalent in patients with CLD (64% to 92%), where they are associated with poor outcomes[112]. Emerging evidence suggests protective effects of vitamin D against hepatic fibrogenesis[113,114]. However, vitamin D supplementation had no beneficial effects in animal models of LC and pre-existing fibrosis[115]. In a recent RCT, vitamin D treatment did raise the serum levels of 25-hydroxyvitamin D in patients with LC, but indicators of liver fibrosis did not improve[116]. The disappointing results can be attributed to the small sample size and short study period, calling for larger and longer studies in the LC population. Nevertheless, LC patients who are vitamin D deficient should receive treatment because LC itself is associated with an increased risk of osteopenia and fracture.

Regenerative therapies and investigational drugs

The potential of cell treatments to improve liver regeneration and modify the course of liver disease has garnered a lot of attention in recent years. There are many different cell types that can be employed to treat LC or fibrosis; however, mesenchymal stem cells (MSCs) are the most often used cell source (73%). Research using animal models have demonstrated that MSC therapy can reduce liver fibrosis, improve liver function, and lessen liver injury[117]. Clinical studies using MSCs for cirrhosis have demonstrated their efficacy in improving liver function; however, large-scale, stratified studies in various CLD settings are required to draw a robust conclusion[117-120]. Also, patients with advanced LC with liver failure, rather than CLC, have been the focus of the majority of clinical trials on stem cells. There is significant heterogeneity between published studies in terms of the type, dose, and method of stem cell delivery, and data accuracy, making it a difficult interpretation[121].

Granulocyte-colony stimulating factor (G-CSF) serves as an alternative to exogenous stem cell infusions by mobilising haematopoietic stem and immune cells. Some RCTs on G-CSF have reported improved survival in patients with ACLF [122,123]. However, in a recent multicentre, open-label, RCT, G-CSF with or without haemopoietic stem cell infusions did not improve liver dysfunction or fibrosis in patients with CLC. Moreover, these medicines were associated with a higher frequency of unfavourable events[124]. Therefore, because of conflicting results, G-CSF is not currently advised for use in CLC patients.

Recent developments in important pathophysiologic pathways linked to PHT in LC have revealed a number of novel possible treatment targets. These include intrahepatic abnormalities associated with inflammation, fibrogenesis, and microvascular changes[57-59,81,125-128]. Some medications, including phosphodiesterase-5 inhibitors, farnesoid X receptor agonists, endothelin-A receptor antagonists, and amino sulphonic acid taurine, have been found to lower PHT, which may be useful in reducing decompensation in CLC patients[125-128]. However, more solid and consistent evidence is required regarding the safety and effectiveness of these medicines. Currently, there are no pharmacotherapies for fibrosis that have been licensed, but research on antifibrosis medications has made significant strides in recent years, especially with regard to medications for nonalcoholic fatty liver disease-related fibrosis[129]. It seems that treatment with a single medication may not be sufficient to treat advanced liver fibrosis due to the complexity of the hepatic fibrosis process, which involves interactions between many cell types including immune cells, hepatic stellate cells, and hepatocytes. Therefore, more studies are needed to determine combination therapies using drugs that have several modes of action.

Elimination of precipitating factors

Several precipitating events can lead to abrupt worsening of clinical condition of LC patients by causing ACLF[130,131]. Furthermore, CLC patients experience a more severe form of ACLF than patients with prior decompensation episodes. Thus, controlling such precipitating variables can thereby significantly reduce cirrhosis-related morbidity and mortality. Antibiotic prophylaxis and prompt, judicious antibiotic treatment can aid in the prevention of ACLF triggered by infection[132]. Prophylactic antibiotics in conjunction with effective gastrointestinal bleeding management can prevent precipitating ACLF.

Another important preventive strategy is vaccination for viral hepatitis. For all LC patients, hepatitis B vaccine is advised. However, compared to normal subjects, patients with cirrhosis achieve lower seroprotection rates following HBV vaccination (mean response rate of 47%)[133,134]. Hepatitis E virus (HEV) and hepatitis A virus (HAV) superinfection is another well-known cause of ACLF in endemic areas[135]. While many nations recommend HAV immunisation for CLD patients, routine vaccination is not advised in some areas, such as India, where the prevalence of HAV antibodies in CLD is apparently greater than 90%. Recombinant HEV vaccines have also been developed but are not

widely available or approved across the world[136]. When vaccination is not an option, general preventive measures like improvement of sanitary conditions and the provision of clean water might decrease the incidence of HEV/HAV-induced ACLF.

CONCLUSION

The life expectancy of patients with CLC is often high, and many of them may be candidates for cirrhosis regression. Therefore, to facilitate the early detection of CLC, medical professionals should employ noninvasive techniques in all CLD patients. Early diagnosis of CLC offers the opportunity to treat underlying causes and prevent or halt the progression of liver disease. For viral cirrhosis, and to a lesser extent non-viral cirrhosis, fibrosis regression may result in cirrhosis reversal when the underlying cause of CLC is treated before progression. The transition from compensated to decompensated cirrhosis is mainly driven by PHT. This transition is accompanied by a dramatic decline in the median survival rates. NSBBs are currently the cornerstones of treatment for PHT, although a number of emerging therapies may pave the way for tailored multimodal strategies in the future. Some more recent drugs have shown promise in decreasing PHT, but more reliable and consistent data are needed to determine their safety and efficacy in LC patients. Furthermore, there are several known risk factors and triggering events for the decompensation of CLC that need to be taken care of. Regardless of the aetiology of CLC, evolving disease-modifying approaches might lead to a paradigm change and a decrease in the burden, morbidity, and mortality associated with LC.

FOOTNOTES

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Country/Territory of origin: India

ORCID number: Ramesh Kumar 0000-0001-5136-4865; Sudhir Kumar 0000-0001-7117-1382; Sabbu Surya Prakash 0009-0006-5117-5443.

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REVIEW

Telemedicine in inflammatory bowel diseases: A new brick in the medicine of the future?

Antonietta Gerarda Gravina, Raffaele Pellegrino, Tommaso Durante, Giovanna Palladino, Rossella D'Onofrio, Simone Mammone, Giusi Arboretto, Salvatore Auletta, Giuseppe Imperio, Andrea Ventura, Mario Romeo, Alessandro Federico

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Antonietta Gerarda Gravina, Raffaele Pellegrino, Giovanna Palladino, Rossella D'Onofrio, Simone Mammone, Giusi Arboretto, Salvatore Auletta, Giuseppe Imperio, Andrea Ventura, Mario Romeo, Alessandro Federico, Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Naples 80138, Italy

Tommaso Durante, Mental Health Department, "S. Pio" Hospital, Benevento 82100, Italy

Corresponding author: Antonietta Gerarda Gravina, MD, PhD, Assistant Professor, Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Via L. de Crecchio, Naples 80138, Italy. antoniettagerarda.gravina@unicampania.it

Abstract

Inflammatory bowel disease (IBD) is a chronic digestive disease that requires continuous monitoring by healthcare professionals to determine the appropriate therapy and monitor short-term and long-term complications. The progressive development of information technology has enabled healthcare personnel to deliver care services to patients remotely. Therefore, various applications of telemedicine in IBD management have evolved, including telemonitoring, teleconsulting, teleducation, telenursing, telenutrition, and telepathology. While evidence has been provided for some telemedicine applications, targeted studies are still required. This review summarises the major studies that have evaluated telemedicine and its application in the management of IBD.

Key Words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Telemedicine; Telemonitoring; Telenutrition; Telepathology; Teleducation; Telepsychology

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Core Tip: The progressive development of technology has provided new telehealth tools for gastroenterologists to manage patients with inflammatory bowel disease. Through online platforms, simple e-mails, phone calls, and websites, physicians can monitor patients, adapt therapies, summon patients in case of alerts or red flags, and communicate with them. However, there is a need to scientifically test these methods by comparing them with the standard of care to determine whether these forms of care are superimposable, or at least comparable.

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INTRODUCTION

Inflammatory bowel diseases (IBD) include ulcerative colitis (UC) and Crohn's disease (CD). They are characterised by chronic, self-sustained digestive inflammation with a relapsing and remitting course that leads to disability, reduced quality of life (QoL), and increased healthcare costs[1].

At present, the exact etiopathogenesis of IBD remains to a large extent, unclear. However, it is believed that it may be the product of a series of complex interactions between genetic (as well as genomic and epigenomic) factors related to the colonic mucosal immune system and gut microbiota. This creates an imbalance in gastrointestinal inflammatory homeostasis by activating the inflammatory process and upregulating proinflammatory cytokines at the expense of anti-inflammatory cytokines[1]. IBD has a significant epidemiological burden. The incidence and prevalence of IBD are steadily increasing, with the highest incidence rates in Western and Northern Europe and North America. The most frequent age range at diagnosis is 16–35 years, and about 6%–7% of newly diagnosed patients are children younger than 15 years[2].

The chronic and relapsing nature of IBD poses the problem of inducing and maintaining remission to avoid developing short-term and long-term complications, disability, and the onset of neoplasia[3,4]. To this end, it is mandatory to continuously monitor various outcomes (*e.g.*, clinical, biochemical, and endoscopic) to weigh the therapeutic efficacy in both the initiation and maintenance phases of treatment to reduce the recurrence rate and minimise complications[5]. Such monitoring is traditionally performed during regular outpatient visits, determination of hematochemical indices, and instrumental investigations, mainly endoscopic exams[5].

However, there is an increasing need to initiate a digital transition in IBD medicine to achieve multiparametric clinical benefits, ranging from increased therapeutic adherence to close monitoring with feasibility in terms of economic sustainability and reduction in healthcare costs[6,7].

The gradual shift from symptom-oriented to prevention-oriented approaches has promoted and accelerated the development of mobile health technologies with the potential to radically transform healthcare delivery[8].

Despite the availability of various digital health tools, there are still no specific codified recommendations regarding which telehealth tools are the best for patients with IBD, the precise indications and contraindications, or the outcomes they can weigh most effectively and safely.

This Narrative Review aims to provide primary evidence on telemedicine applications, specifically in the setting of IBD.

TELEMEDICINE: GENERAL CONSIDERATIONS

Telemedicine: Definitions and applications

Telemedicine uses technological tools to provide medical care (from diagnosis to treatment) from a distance[9].

The need for a proper definition of telemedicine is challenging in the early stages of testing and implementation. More than fifteen years ago, a peer review of one hundred and four different definitions of telemedicine allowed telemedicine to be defined as "Telemedicine being a subset of telehealth, uses communications networks for delivery of healthcare services and medical education from one geographical location to another, primarily to address challenges like uneven distribution and shortage of infrastructural and human resources"[10].

Several aspects must be addressed when approaching the subject of telemedicine. First, while it offers the possibility of providing care with greater immediacy and constancy, and at a potentially better cost-benefit ratio, there are several aspects to be considered. Specifically, those related to privacy, confidentiality, and the need to achieve adequate information transparency with prior informed consent well-stated by the patient have already been stigmatized[9].

Telemedicine is not a particularly recent technique, but pioneering examples were found even before the 2000s. The telepsychiatry system was established in 1959 at the University of Nebraska School of Medicine[11]. However, the modern era of telemedicine occurred later in 1968 when a multispecialty telemedicine system was established at Massachusetts General Hospital and offered to workers and passengers at Logan International Airport[11].

Examples of national telehealth programmes can be identified later, in the 1980s, by the Norwegian government to provide health services to isolated portions of the population in rural areas with difficult access to health care. Similar services were offered in Australia, Canada, Japan, and the United States[12]. Since the 1990s, telemedicine has undergone marked growth in the technologies used and territorial implementation[11].

Bashshur *et al*[13] presented a telemedicine taxonomy by identifying three dimensions (functionality, application, and technology). Functionality, in turn, is divided into consultation, diagnosis, mentoring, and monitoring applications, and into specialty, disease, site, and treatment. Finally, technology was divided into synchronicity, networks, and connectivity. This is called the "three-dimensional model"[13].

Synchronicity is an important term in the telemedicine glossary in that telemedicine can be carried out as much in an "asynchronous" sense as in the case of diagnostic images or surveys as in a "synchronous" mind, for example, in remote medical examinations[14]. The need for greater connectivity has increased dramatically in recent decades. This can be partly explained by the extensive advances in digital technology, which have made it increasingly easy to connect patients and healthcare providers remotely[14]. Easy access to scientific sources also lays the foundation for continuing medical education at a distance[12]. This could also be relevant in the field of IBD, given the continuous progress made in medical and surgical science in this field.

Telemedicine offers several benefits. The first is the possibility of improving access to health services and increasing the spread with which these are delivered[12]. Second, it is possible to enhance the degree to which patients and healthcare providers are informed while setting up systems for quality control and improving the feasibility of screening programmes[12].

As telemedicine should affect IBD, we need to consider that it is a chronic condition with a very often unpredictable course and epidemiology that deeply affects younger populations[15]. Telemedicine can interject itself into these difficulties and provide support in monitoring the disease (telemonitoring), providing medical care and choices (teleconsulting), educating patients about IBD and its management (teleducation), and managing the nutritional aspects (telenutrition) (Figure 1).

Already two meta-analyses on telehealth in IBD have shown that current evidence may justify a role for it in the treatment of IBD[16,17]. This finding seems to be most evident for QoL in adolescents and in the reduction in the number of outpatient visits[16].

The patient's point of view: Perspectives and beliefs related to telehealth

Regardless of the remote care technique employed, IBD patients must benefit and accept it. Several studies before and after the coronavirus disease 2019 (COVID-19) pandemic weighed the expectations and perspectives of IBD patients in this regard. However, while there are benefits of non-invasive and continuous healthcare, there is also a need to ensure equity in the delivery of telemedicine and assurance of confidentiality[18]. Therefore, the voice of the patient, the recipient of these services, must be carefully heard.

A systematic review conducted by Al Khoury *et al*[19] identified that the highest expectations experienced by patients with IBD were pain control, endoscopic reporting in the normal range, and adequate QoL. Additionally, some patients wanted to be informed by gastroenterologists about their IBD. However, one of the most interesting elements that emerged was the propensity of patients toward e-health tools that were deemed feasible and acceptable.

Beyond healthcare, e-health resources are also potential sources of information for patients with IBD. For example, an extensive survey involving more than 300 patients with IBD revealed that, while the healthcare team was the preferred source of medical information, the second favourite source was the Internet[20]. Approximately 80% of the participants had searched the Internet for information about IBD, and approximately 30% did so at least once a week. In this study, patients were given a website for consultation, as recommended by health professionals.

This survey provides a source of food for further research. First, there probably needs to be an effort by health professionals to alert patients to potential sources of misinformation on the Web. Indeed, the concept of "infodemic" to which patients are subjected has emerged with COVID-19 more and more strongly[21].

Several surveys were conducted during the pandemic to weigh the beliefs and perceptions of physicians involved in managing IBD and patients suffering from healthcare consequences.

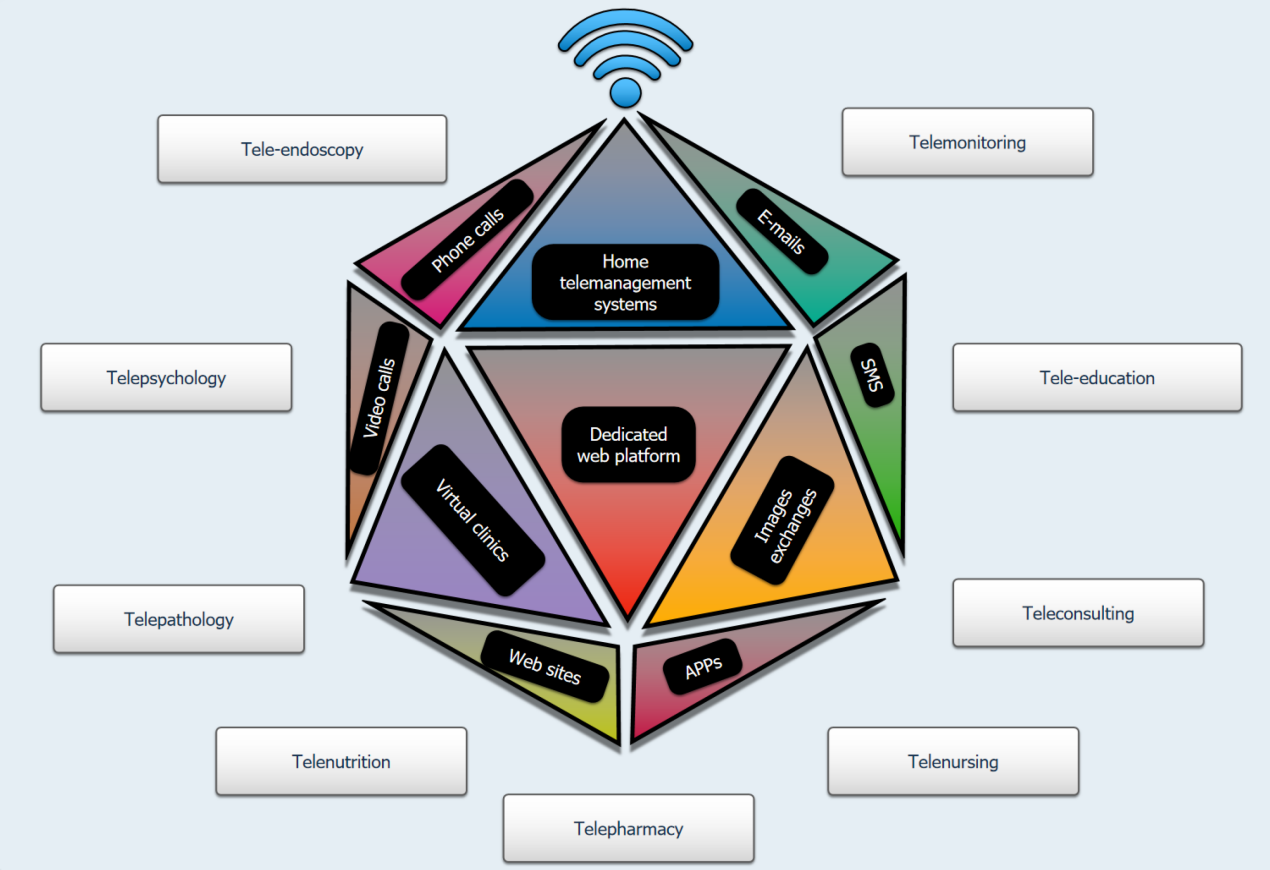
Patient beliefs regarding the relationship between IBD and COVID-19 are among the major issues to be addressed by busy gastroenterologists during the pandemic.

For example, a Portuguese survey recorded how patients believed they were at an increased risk of severe COVID-19 if they had active IBD or were taking corticosteroids within three months[22]. In addition, Pellegrino *et al*[23] reported a reduction in therapeutic adherence during outpatient follow-up during the COVID-19 pandemic at a referral IBD centre in southern Italy by employing a remote questionnaire-based interview.

Additionally, in an Indian survey, patients reported similar fears aimed at patients in remission in most or all-controlled clinical activities in which telemedicine methods had also been applied in most of the sample[24]. The authors reported reduced involuntary adherence (due to the unavailability of medication, financial constraints, and difficulty in reaching dedicated health facilities) with increased disease activity. In addition, the authors recorded an involuntary need to switch therapies due to the unavailability of previous therapies.

In addition, a French survey examined how telemedicine met with some success in terms of preference by healthcare personnel and a sample of patients during the COVID-19 pandemic[25]. However, an element that emerged in this survey was the need for a filter because disease flare-ups require in-person consultation.

Indeed, one cannot analyse the changes forced by the restrictions of the pandemic on remote medicine systems without considering that not all age groups have overlapping adaptive capacities. Moum *et al*[26], for example, showed in an analysis of more than 500 patients with IBD that although the preferred follow-up method was outpatient visits, the under-50 age group preferred telephone follow-up more than the older age group. Unsurprisingly, another study showed



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Figure 1 Telehealth modality proposed in the management of inflammatory bowel diseases. Various data-obtaining and telecommunication systems can be established between patients and physicians engaged in the direction of inflammatory bowel diseases (IBD) management. Combining these data and interactions leads to the genesis of various telemedicine applications in the IBD setting to ensure a range of outcomes (*i.e.*, clinical, therapeutic, diagnostic, nutritional, psychological, *etc.*).

that younger patients were more prone to use telemedicine[27]. However, other evidence has shown encouraging results regarding the involvement of older adults in telemedicine systems, especially with the advent of the COVID-19 pandemic [28].

Another system by which patients with IBD can receive remote healthcare is through social media (*e.g.*, Facebook, Instagram, LinkedIn, Twitter). A survey conducted in 2020 on over 100 patients with IBD (with an average age of 47 years) showed that Facebook and Instagram were the social networks most frequently used by patients. Of these, approximately 30% used social media concerning their IBD (*i.e.*, for information about IBD, for support and coping strategies, to improve their anxiety levels, and to connect with other IBD patients)[29]. In addition, most participants (72.3%) reported wanting to receive telemedicine through society.

The use of social services for this purpose was also evaluated in settings where healthcare became complex, that is, in rural locations. For example, an experiment conducted in the rural community of West Virginia involving over 600 patients with IBD confirmed that Facebook and Instagram were the preferred social platforms, and over 90% of the sample desired to receive care from their physicians through them[30].

Ultimately, different voices emerged in different geographical settings, with a common matrix of good acceptability/preference for telehealth tools by IBD patients.

The COVID-19 pandemic helped accelerate the implementation of telemedicine in the management of IBD: From pandemic damage to the possibilities of remote medicine

In 2019, the outbreak of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a beta-coronavirus, arose in the Wuhan district of China and gradually acquired characteristics defined as a pandemic by the World Health Organization [31]. Although the diction of SARS-Cov-2 emphasizes the primary pulmonary involvement of the disease, it has gradually emerged that the condition could also affect the gastrointestinal tract as much as nonspecific clinical manifestations (such as vomiting/nausea, abdominal pain, and diarrhoea), as with direct organ damage as in hepatitis[32]. Furthermore, gastrointestinal involvement is associated with a worse intrahospital prognosis and requires intensive care[32]. Nevertheless, severe gastrointestinal disorders related to COVID-19 have been reported[33-35]. Additionally, suspicions have been raised regarding the possible role of SARS-CoV-2 in triggering the onset of *de novo* IBD[36]. The gastroenterological world has been strongly advocating preserving patients with IBD and those who are chronic and require continuous care over time. Telemedicine has accelerated abruptly to address this need[37]. The COVID-19 pandemic has

drastically occupied beds in hospital facilities, resulting in the closure of outpatient facilities for emergency management and the reshuffling of healthcare personnel by redirecting them to COVID-19 centres. Therefore, the need for telemedicine arose from an unquantifiable set of requirements for IBD patients, impacted by the priority that COVID-19 patients had acquired (such as management of moderate-severe cases, severe acute UC, complicated CD, chronic control of biologic therapy, and oncologic surveillance of patients with long-standing IBD)[38].

Telemonitoring during pandemics has often been mandated in territories affected by lockdowns. Nevertheless, an economic analysis has also suggested telemonitoring as a cost-effective strategy for improving quality-adjusted life-years and costs of care[39].

All these events have, in the gastroenterology research landscape, resulted in substantial growth of studies examining the potential of telehealth in the IBD patient (Figure 2).

TELEMONITORING IN IBD

Definitions

Medical telemonitoring is a continuous or non-continuous monitoring process generally applied to chronic diseases, allowing healthcare providers to monitor and interpret patient data to make decisions about the health of the monitored patient[40]. Over time, several modalities have been outlined for applying information technology to telemonitoring for IBD. For example, some studies have examined home tele management systems and website-based systems as the most straightforward systems for the exchange of emails or phone calls between physicians and patients[41].

Many studies conducted on telemonitoring also included telemonitoring components because they provided information and educational content to patients about their IBD[42,43].

Cross group's experiences with the home automated tele management system

The United States Cross Group evaluated several applications of the home automated tele management (HAT) system for IBDs in subsequent studies. The HAT system consists of three elements: A patient unit, a server supporting decision-making, and a web portal for physicians[44]. However, this system has not been applied to many patients with IBD. The first component was a laptop that served patients for self-testing. This concept is based on the genesis of alerts exhibited to clinicians (*i.e.*, alert systems) from a dedicated web system when the patient's self-reported values exceed certain predetermined thresholds. The main variables collected by the authors were based on a numerical scale to weigh symptoms, compliance with medical therapy, body weight, and adverse events related to treatment.

In 2006, the first uncontrolled and nonrandomised pilot study enrolled five patients with CD and five with UC (almost all Caucasians) with a mean age of approximately 48 years. None of the patients exhibited severe disease activity. Several interesting findings emerged from the study, including the fact that 20% of the participants had not been previously educated about computer use. All patients found HAT feasible and easy to use without a special commitment to their daily lives by ensuring weekly completion[44].

The same group conducted a subsequent HAT-based study in 2007 on 25 patients with IBD, with a mean age of 43 years. In this study, the feasibility and patient acceptance of HAT re-emerged. During the study, which took place over six months of follow-up, there was a reduction in disease activity and indices of inflammation (*i.e.*, C-reactive protein and erythrocyte sedimentation rate). This was also accompanied by an increased QoL and patient self-awareness of IBD[45].

The authors also evaluated patients' preferences for HAT by confirming their acceptability in a survey of patients included in HAT studies[46].

Finally, the cross-group conducted a randomised controlled trial on HAT targeting 25 UC patients on HAT *vs* 22 on the standard of care with a follow-up in both groups of one year[47]. However, during the study, 11 patients dropped out of the HAT group, and at the end of the follow-up, the authors concluded that there was a lack of reduction in disease activity as well as a failure to increase therapeutic adherence. Nevertheless, corrective analyses revealed a modest increase in QoL. However, this study was limited by its small sample size, which might have revealed only gross differences between the groups; the fact that there was a significant proportion of patients in long-term remission; and the dropout rate in the intervention group.

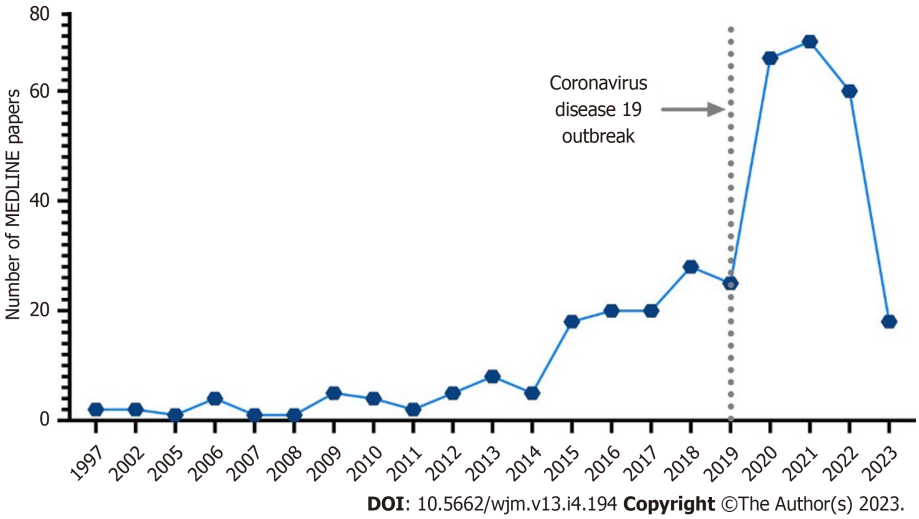
Ultimately, this system showed excellent patient acceptability and feasibility in all studies. However, as described, the data on QoL and impact on therapeutic compliance and disease activity were discordant and inconclusive, probably because of the small sample size common to all three studies.

However, these experiences, while home-based, have been among the starting points for more modern expressions of telehealth and do not require special hardware devices to be placed in patients' homes.

The gradual evolution toward more immediate systems: The "Constant-Care" web-based telemonitoring

The rapid and recent evolution of the Internet has resulted in the development of numerous online computer platforms, from real-time chat systems and emails to online portals dedicated to health. Thus, the concept of eHealth (the use of telecommunications and information technology in healthcare services) emerged. The concept of eHealth has been introduced for the development of portable medical devices.

A Danish group employed a web-based telemonitoring system called Constant-Care embedded explicitly in a dedicated website (www.constant-care.dk), validated in an initial study conducted over a decade ago in 21 patients with mild-to-moderate UC[48]. The authors assessed various outcomes from education (*i.e.*, knowledge of IBD), QoL, anxiety, and depression levels. While no particular impact was recorded on the QoL and mood (*i.e.*, anxiety and depression), the systems showed excellent feasibility and acceptance by patients and increased knowledge of their disease. The same



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Figure 2 Trends in telehealth research before and after the coronavirus disease 2019 outbreak. Conducting a simple search in papers indexed in MEDLINE, it is glaring how since 2019 (the year in which the onset of today's coronavirus disease 2019 pandemic falls), there has been significant growth in papers produced combining telehealth with inflammatory bowel diseases (IBD). We also note, predictably, that before 2019 the mass of papers on IBD gradually showed a gradual growth trend in the period 1992-2008. The search for such data in this figure stopped on 2 April, 2023.

group later conducted a randomised controlled trial on the same platform, including over 300 patients with mild-to-moderate UC on first-line therapy with 5-aminosalicylate acid derivatives, randomised into two groups (constant care *vs* usual care) for one year[49]. In contrast to the first study, in addition to the confirmation of feasibility/acceptance by patients, there was an increase in therapeutic adherence and QoL compared to the control and a decrease in the number of outpatient visits and the average patient/year costs. However, there was no impact on the frequency of IBD flare-ups, hospitalisation, or surgical outcomes.

Moreover, the Constant-Care platform has been used to optimise infusion sessions in patients with CD undergoing intravenous therapy with infliximab. In detail, Pedersen *et al*[50] in an initial pilot study included 27 patients with CD on maintenance infliximab therapy for 52 wk of follow-up (reached in the majority of the sample). The authors recorded the platform data regarding symptomatology and CD activity of the patients weekly, and faecal calprotectin levels were recorded weekly. All these parameters were computed into a score (*i.e.*, inflammatory burden score) adopted by the authors to classify patients into a visual colour system (green, yellow, or red zones) to refer yellow and red patients for medical consultation. An interesting element emerging from this pilot study was the high rate of therapeutic change: 39% of the sample obtained therapeutic optimisation, while 50%, on the contrary, achieved longer intervals between infusion sessions. Pedersen *et al*[51] applied the same platform in a prospective open-label study targeting 95 patients with mild-to-moderate UC on mesalazine therapy for three months showing excellent results on the therapeutic adjustment of its dosages. Patients were required to complete the simple clinical colitis activity index each week and their faecal calprotectin values were recorded. Again, the authors, employing these variables, calculated an equal inflammatory burden score, similar to what was done in a previous study of CD patients. There was a significant reduction in the simple clinical colitis activity index score and faecal calprotectin levels. Through this form of monitoring, the authors reduced the dosage of mesalazine in a quarter of the patients at week 3, in half of the subjects at week 5 and in almost all patients (88%) at week 12.

Subsequently, Carlsen *et al*[52] applied a monthly Constant-Care web application (www.young.constant-care.com) to paediatric patients with IBD, significantly reducing outpatient visits and school absences in the constant-care group compared to the control group, which followed classic quarterly outpatient monitoring. There were no significant differences in therapeutic adherence or QoL between the intervention and control groups. An interesting finding was the excellent acceptability (81% adherence) in a complex setting, such as paediatrics.

Other experiences based on telemonitoring systems using web-based systems

Other web-based portals have been implemented to establish telemonitoring for patients with IBD. For example, de Jong *et al*[53], in 2017, examined a telemonitoring system (myIBD Coach) in a randomised trial. This system is a webpage accessible from tablets and smartphones for the monthly monitoring of disease activity levels, therapeutic adherence, adverse drug events, nutritional status, anxiety and depression levels, and other outcomes (such as physical activity and patients' ability to self-manage). Physicians can monitor these parameters, communicate with patients, and use the e-learning modules. The authors assigned more than 450 patients to the myIBD Coach group and 444 to the usual care group. The study's primary results were a significant reduction in hospital access for outpatient visits and the number of hospitalisations in the myIBD Coach group.

A further randomised trial compared the web-based system on the platform "Telemonitorización de la Enfermedad de Crohn's Disease y Colitis Ulcerosa or Telemonitoring of Crohn's Disease and Ulcerative Colitis" (TECCU) *vs* usual care and telemedicine by phone calls by examining clinical outcomes (*i.e.*, clinical remission at week 24, QoL, therapeutic adherence, adverse events to therapies, social activities, hospitalisations, IBD surgery, number of outpatient visits)[54]. In

these three study arms (21 patients per group) consisting of patients on immunosuppressive therapy, the TECCU group had the highest remission rate, the best disease activity scores, and reduced faecal calprotectin values.

Cross *et al*[55], to overcome the limitations of the HAT system, also evaluated another web-based telemedicine system [*i.e.*, telemedicine for Patients with IBD (TELE-IBD)] based on text messages. A three-arm randomised trial (TELE-IBD administered every week, TELE-IBD every two weeks, and a standard care group) involved more than 300 patients with IBD with at least one disease flare-up tracked in the previous two years. The trial showed improved disease activity and QoL, and reduced hospitalisations in the TELE-IBD group; however, no clear superiority of TELE-IBD over the standard of care was demonstrated. Schliep *et al*[56] conducted a follow-up analysis of the data from the already mentioned TELE-IBD trial by Cross *et al*[55], who conducted a specific analysis on the levels of depressive symptoms (assessed with the Mental Health Inventory 5) and QoL (set using the Short Form 12) and showed that text message-based monitoring did not increase depressive symptoms and QoL.

A trial based on web-based telemonitoring with text messages was conducted by Bilgrami *et al*[57] in 222 IBD patients with at least one exacerbation in the previous two years compared with usual care. Again, no particular difference emerged in the outcomes assessed between the telemonitoring and standard of care groups (*i.e.*, self-efficacy and patient activation).

An additional platform in this context is the EasyMICI-MaMICI® studied in a randomised controlled trial directed at patients with active IBD *vs* standard of care and showed improved QoL and satisfaction in the included patients (*i.e.*, 54 IBD patients) and reduced outpatient visits[58]. EasyMICI-MaMICI® includes a smartphone-accessible app (EasyMICI) and online portal system (MaMICI). This platform provides a method for data collection and enables communication between users and caregivers.

Ghoshal *et al*[59], in 2021, employed a web-based platform video/audio consultation during the COVID-19 pandemic in 50 IBD patients reviewing IBD activity, anxiety levels, QoL, and probable COVID-19 symptoms, and recorded a comparable SARS-Cov-2 infection rate in IBD *vs* non-IBD controls, and QoL comparable or better than before the pandemic.

Ultimately, web-based systems have been among the most abundantly tested and have provided interesting results, as described above, regarding first-level outcomes in IBD (*i.e.*, clinical remission rates and hospitalisations).

More accessible telemonitoring systems: Use of phone calls, text messages, e-mails or apps

Telehealth strategies that do not use complex online platforms or home-based telemonitoring systems should certainly be included in the analysis of telemonitoring, as well as in systems that have tools within everyone's reach, such as simple short message service messages, emails, or even simpler phone calls between healthcare caregivers and patients with IBD.

Examples of such methodologies can already be found several years ago. In 2009, a retrospective study was conducted by Torrejón Herrera *et al*[60], who aimed to collect care data from the Crohn's Colitis Care Unit at Vall d'Hebron University Hospital in Spain over nine years (*i.e.*, 1999 to 2007). They collected data from 1784 patients and observed how remote tools (telephone and fax) were employed more often than presential activities. The Crohn's Colitis Care Unit also used a web-based system (which recorded over 3500 registered IBD users and over 150000 visits).

Other examples can be traced to the paediatric population. For instance, Heida *et al*[61] telemonitored 160 patients aged 10 to 19 years through the use of e-mail and phone calls. They found an increase in the patients' self-efficacy and acceptance of telemedicine. The authors also recorded significant economic gains and reduced the number of in-person visits in the telemonitoring group.

Pinto *et al*[62] telemonitored 21 patients with CD and 56 patients with UC *via* telephone calls during the COVID-19 pandemic through a collection of patient-reported outcomes to assess disease activity and adopt treatment adjustments.

Few studies have examined cell phone applications as tools for IBD settings. Among them, a study conducted by Echarri *et al*[63] evaluated the Harvey-Bradshaw index self-administered *via* an app (*i.e.*, the MediCrohn Study) by patients to determine if their results agreed with the same index calculated in the hospital by a physician. The study found a high percentage of concordance between the Harvey-Bradshaw index assessed by the app and that assessed by the physician. Another study by McCombie *et al*[64] using a telemonitoring system for outpatients employing two smartphone apps (*i.e.*, "IBDsmart" for symptoms and "IBDoc" for faecal calprotectin monitoring) and comparing it with usual care leaned toward the efficacy and acceptability of the telemonitoring system finding it particularly effective when used in patients with mild to moderate disease not recently diagnosed with a reduction in outpatient visits. Similar results were reported in a randomised trial by Östlund *et al*[65]. Again, participants found self-monitoring using a home faecal calprotectin assay and a digital application feasible and appreciated.

Finally, the study conducted by Zhen *et al*[66] involved the use of an app (*i.e.*, "HealthPROMISE") by patients with IBD to monitor the condition after one year of data collection showed no improvements in QoL between patients followed through the app and those undergoing standard care; however, patients reported an increase in their understanding of the nature/causes of their condition after the period of using the app, a statistically significant decrease in IBD-related hospitalisations and emergency room visits was also found.

TELECONSULTING IN IBD

Teleconsulting provides healthcare consultation *via* telecommunications, directed by healthcare personnel to patients. Therefore, many of the aforementioned studies on telemonitoring inevitably have a teleconsulting component. In addition, many of the telemonitoring systems and platforms studied are aimed at providing consultations to patients to make diagnostic or therapeutic choices. However, over time, experiences aimed at studying teleconsulting (in isolation),

its impact on clinical practice, and the significant outcomes of IBD have emerged.

A preliminary study conducted by Krier *et al*[67] used a real-time imaging-based system (*i.e.*, Solar Digital Unity software, San Diego, CA, United States) capable of integrating audio-visual communication between physicians and patients as well as the ability to share digital content (such as diagnostic images) to care for 34 veterans with IBD. The authors demonstrated that, through this follow-up system of telemedicine outpatient consultation *via* video calls, a similar level of patient satisfaction was achieved as that obtained from in-person encounters concerning indices such as attention to patient concerns and the physician's perceived skill level. The interviewed physicians welcomed the technical and informational qualities of the telemedicine sessions.

In addition, in the retrospective experience of the Crohn's Colitis Care Unit at Vall d'Hebron University Hospital in Spain by Torrejón Herrera *et al*[60], much of the care provided to patients over the nine years of reviewed care was *via* teleconsulting.

In the Highlands and Scottish Isles, the study conducted by Ruf *et al*[68] showed that Virtual Care clinics can be a safe and effective model of patient-centred care for patients with IBD living in remote areas, allowing enormous potential for time and cost savings.

Li *et al*[69] evaluated whether a telemedicine-based IBD clinic could provide a high-quality, low-cost alternative to traditional care. Telemedicine clinics are based on virtual appointments with IBD specialists using an Internet-connected device. Patients completed a pre-visit survey reporting information about their disease activity, a post-visit study about their experience during the web appointment with the specialist, the time and money saved by not having to travel to the visit, and their preferences for future visits. At the end of the study, 77% of the patients reported that they preferred web appointments, again demonstrating the applicability of web monitoring systems as an alternative to outpatient visits to save time and money without compromising quality of care.

Gastroenterologists and IBD specialists can also benefit from the educational potential of telemedicine as it provides a forum to discuss patient cases with complex diagnostic or treatment dilemmas. Live inter-institutional interdisciplinary videoconference education (IBD LIVE) is an example of a multisite, multidisciplinary videoconference platform. The participating members included gastroenterologists, surgeons, pathologists, radiologists, and other medical specialists. The objectives of each session included a review of evidence-based data and exchange of inputs for managing patients with IBD[70].

TELENUTRITION IN IBD

IBD causes significant digestive changes that require careful evaluation and monitoring by clinical nutritional specialists. Therefore, attention has also been paid to telemedicine (although few studies are available) for the possibility of providing nutritional care to patients with IBD remotely (telenutrition).

In addition, the nutrition of patients with IBD must be adapted not only to the patient's clinical-demographic and anthropometric characteristics, but also to changes in disease activity in situations in which therapeutic fasting and parenteral nutrition may be necessary[71].

While many of the studies conducted in telemedicine have primarily focused on modifying medical-therapeutic parameters for outcomes directly related to IBD disease activity, another concept is also emerging. Given the lack of need for a physical examination in many dietitian encounters, telenutrition could fit into this context and address the nutritional needs of IBD patients[72].

Similar to the previously discussed telemedicine applications, the nutritional aspect has also been dramatically affected by the COVID-19 pandemic, and there have been many efforts to provide chronic patients with remote nutritional care [73,74].

Thus, telenutrition can investigate various aspects such as weight history, food intolerance, diet setting, evaluation of diet response, hunger assessment, monitoring, and therapeutic adjustments of enteral and parenteral nutrition therapy [75]. However, in the available studies, the study design was not always explicitly outlined for the remote assessment of nutritional status. This outcome is often one of the other outcomes examined (*e.g.*, disease activity and therapeutic adherence).

For example, Ehrlich *et al*[76], in 2012, proposed an app (*i.e.*, "GI Buddy") available for iPhones or also *via* a dedicated website in which patients 13 years of age and older entered data related to patient-reported medical outcomes but also dietary logs. In other words, there was a categorisation of data that the patients could input: Symptoms, treatment, diet, and lifestyle.

Later, there was a nutritional component in a previously described study by de Jong *et al*[53] employing the myIBD Coach app. A teleducation service also provides educational modules for nutrition and IBD.

Other teleconsulting experiences (*e.g.*, the Promoting Access and Care through Centres of Excellence Telemedicine Program) included the nutritionist experience in IBD as part of the team and consultation[77].

An Indian congressional communication at the 17th Congress of the European Crohn's and Colitis Organization presented a digital health platform designed explicitly for telenutrition (*i.e.*, IBD NutriCare)[78]. This application, available for both Android and iOS, allows the recording of dietary variables based on over 600 Indian recipes, as well as other clinical parameters of disease activity. These data were then used to analyse the nutrients consumed by patients to provide real-time tracking of the diet of the monitored IBD patients.

In conclusion, evidence for telenutrition in patients with IBD is particularly scarce and has not increased, even during the COVID-19 pandemic. Therefore, more efforts from the research community on IBD and nutrition are undoubtedly desirable to test new tools and further validate those already available to improve chronic nutritional care and properly

manage patients with IBD.

TELEDUCATION IN IBD

There are not many studies conducted exclusively on teleducation. Instead, many of the studies conducted in other branches of telehealth have included teleducation components. Some examples are the telenutrition platforms explained above. As stated, de Jong *et al*[42,53] included in the "MyIBD Coach" platform specific e-learning modules delivered to patients with IBD. Central among these modules was the discussion of IBD in general and the importance of immunosuppressive and biological therapies and traditional treatments (*i.e.*, mesalamine). In addition, the authors educated patients on "self-management", that is, adopting strategies to prevent/reduce symptoms. Also completing these modules were sections on influenza vaccination, anxiety and depression. As mentioned above, an authentic "educational curriculum" was also provided in the TELE-IBD platform[43]. In this system, each participant received educational advice once or twice a week (depending on which intervention arm they belonged to). In addition, healthcare providers could also send educational messages exempt from this temporal logic concerning, for example, advice during flu seasons. Similarly, the web-based platform "Constant-Care" also hosted educational training on the website[48].

SPECIAL APPLICATIONS

Telepathology

Care for patients with IBD is also achieved through accurate diagnosis of histologic specimens both at the time of the first diagnosis, where differential diagnosis between CD and UC becomes crucial, and during follow-up endoscopic investigations and colorectal cancer surveillance.

In telepathology, a remote consultation with a pathologist is achieved by transmitting digital images. The main telepathology experiments conducted in the field of IBD have primarily targeted the burden of interobserver variability in diagnosing IBD-associated dysplasia/cancer, which often requires evaluation by multiple pathologists.

In the early 2000s, Odze *et al*[79] weighed the utility of telepathology and interobserver variability in detecting UC-associated dysplasia in approximately forty cases of UC. Four pathologists reviewed the slides of the haematoxylin-eosin images of UC. The degree of concordance among the pathologists was fair, with a kappa (κ) of 0.4 and worse results were observed for low-grade or indefinite dysplasia. However, the resolution of the images had not yet reached the current quality levels, so much so that, in this study, the same pathologists had generally given an upgrade in the degree of dysplasia on direct evaluation of the slides, but not in telepathology. The method used to obtain images was a Twin Cam MX-700 digital camera (Fuji film) provided with a (for use with a microscope) ocular device (I. Miller Precision Optical Instruments Inc., Philadelphia, PA, United States).

Later, Odze *et al*[80] recorded telepathology in the same setting involving seven pathologists (of whom only one was an expert in digestive pathology). A dynamic Apollo Telepath system (Apollo Telemedicine, Falls Church, VA, United States) was deployed for image transmission. However, the κ was poor (k = 0.32), with a worse level for low-grade and indefinite dysplasia.

However, subsequent studies have shown more encouraging results. For example, Wu *et al*[81] involved four Chinese pathologists in examining fifty IBD colonic biopsies digitized using the Aperio system (Leica Biosystems). The degree of agreement among the pathologists was much higher (k = 0.68) for interpreting IBD-associated neoplasms. Moreover, an interesting aspect of the study was the comparison of this agreement with that of four pathologists in the United States. In this case, the κ was greater than 0.74.

Further experience was provided by the Italian Group for the study of the Inflammatory Bowel Disease (*i.e.*, IG-IBD) pathology group[82]. In this study, 20 pathologists with experience in digestive pathology evaluated 54 diagnostic blocks from 30 colonoscopies in patients with IBD. Dysplasia detection showed a moderate degree of agreement (κ = 0.48).

Ultimately, telepathology could be of great help in the management of patients with IBD. Guidelines recommend that the evaluation of dysplasia in patients with IBD, given the increased risk of colorectal cancer compared to the general population, should be performed by pathologists experienced in IBD[83]. However, IBD referral centres are not always available in all geographic regions; therefore, telepathology could provide valuable support in such settings.

Telenursing

IBD nurse practitioners are increasingly gaining importance in clinical life[84-87], even in post-surgical stoma management but little evidence is available on telenursing (*i.e.*, remote nursing care provided to patients with IBD)[88].

Cook *et al*[89] reported in 2010 on the application of telenursing to address cognitive and emotional barriers to therapeutic adherence of mesalazine in patients with UC. A large sample of > 200 patients was included in a nurse-managed telephone follow-up programme to increase therapeutic adherence through cognitive-behavioural and motivational techniques. Ultimately, the authors recorded a significant increase in therapeutic adherence after the telenursing programme.

In a study by Del Hoyo *et al*[90], nurses played a role in telemonitoring IBD patients *via* telephone assistance, showing encouraging results from a cost-effective perspective. Specifically, telenursing experience showed a 67% probability of producing economic savings per additional quality-adjusted life-year compared with standard care.

Squires *et al*[91] found that a nurse-operated telephone advice line is a cost-effective intervention that allows patients to avoid going to the hospital when unnecessary and saves money in primary and secondary care.

A similar study by Sanromán Alvarez *et al*[92] found increased telephone consultation requests by nurses and a decreased need for medical consultations, with savings on care amounting to 73.603 euros from 2009 to 2011.

Ultimately, the role of IBD nurses in telehealth has yet to be well evaluated in the literature and could show strong potential in managing chronic patients such as IBD patients and providing relevant support to medical staff. In addition, a large proportion of these studies employed nurses as ancillary elements through phone calls. Studies in which IBD nurses could play a more primary role would perhaps be desirable since they often spend the most time with patients with IBD.

Telehealth as a tool to determine anxiety-depressive disorders in the IBD patient and the initial experiences on telepsychology

It is increasingly emerging that the gut-brain axis plays a role in the pathogenesis of IBD as it affects the course of the disease, such that bidirectional communication with a mutual influence between the brain and gut is realised[93]. The bidirectionality in the gut-brain axis is one of its gnoseological pillars, in that the prevalence of anxiety-depressive disorders reaches about 30% in patients in remission and increases to about 70% in patients with active IBD[94]. During the COVID-19 pandemic, it was also observed in Italy that, during the first lockdown, there were particularly high rates of anxiety, depression, and poor sleep quality in patients with IBD in remission[95]. Nonetheless, anxiety-depressive disorders may also influence the frequency of disease flare-ups in patients with IBD, potentially leading to an increased frequency of flare-ups, an often more aggressive presentation, increased rates of hospital readmission, and an increased risk of surgical intervention[94]. However, this issue is not always adequately addressed in managing IBD and often takes a back seat. Unsurprisingly, some studies have reported that, in some settings, up to 70% of patients with anxiety-depressive disorders (even severe ones) are not treated by a dedicated specialist[96-98].

The different psychological techniques applied to IBD include cognitive behavioural therapy (CCBT), social support, stress management, and targeted techniques[98,99]. Many studies that have weighed the impact of psychology on managing IBD have also included remotely performed treatments.

Therefore, telemedicine and telepsychology interventions could be promising alternatives for patients with IBD who need them. In addition, web-based psychological interventions have proven effective in other situations, such as treating depression and anxiety[100,101], insomnia[102], and irritable bowel syndrome[103,104].

Some of these projects are currently underway. One example is the randomised trial by Evans *et al*[94] known as the "ACTforIBD" program. The latter is based on acceptance-commitment therapy (*i.e.*, a technique specifically for patients with IBD and concomitant psychopathological comorbidities) to address unresolved problems related to chronic IBD. This technique will be administered to patients by dedicated psychological therapists in one-hour sessions for eight weeks.

Schliep *et al*[56] additionally conducted a posthoc analysis of data from the TELE-IBD trial[55]. They extrapolated the variables of mental health (assessed using the Mental Health Inventory 5) and QoL (assessed with the Short Form 12 questionnaire). They compared a group that received telemedicine with a control group (*i.e.*, standard of care). The TELE-IBD method, which is mainly based on text messages, did not positively affect depressive symptoms or the QoL.

Other studies have attempted to provide CCBT psychotherapy to remote patients with IBD by comparing it with the in-person standard of care. For example, McCombie *et al*[105] showed that CCBT is associated with improved disease activity and QoL, including anxiety and depressive symptoms, in the first 12 wk. However, these results were not confirmed at six months. However, patients with less than 50% adherence to the CCBT program had many outcomes at six months. Therefore, future research should focus on adherence to psychological therapies.

Telepsychology has also provided interesting results regarding adherence to conventional therapy for IBD. For example, Hommel *et al*[106] offered behavioural therapy with four 60–90-minute sessions to patients with IBD and measured therapeutic adherence with the pill count strategy; they reported interesting results, although not significant, about a 29% increase in adherence to mesalazine.

Tele-endoscopy

Patients with IBD require recurrent assessment with quality endoscopic methods[107] as much in initial diagnosis as in monitoring and oncologic surveillance, so much so that endoscopic outcome is the focus of the most up-to-date "Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE-II)" consensus[108].

To consider the application of telemedicine in endoscopic evaluations, one can turn to the concept of “tele-endoscopy”.

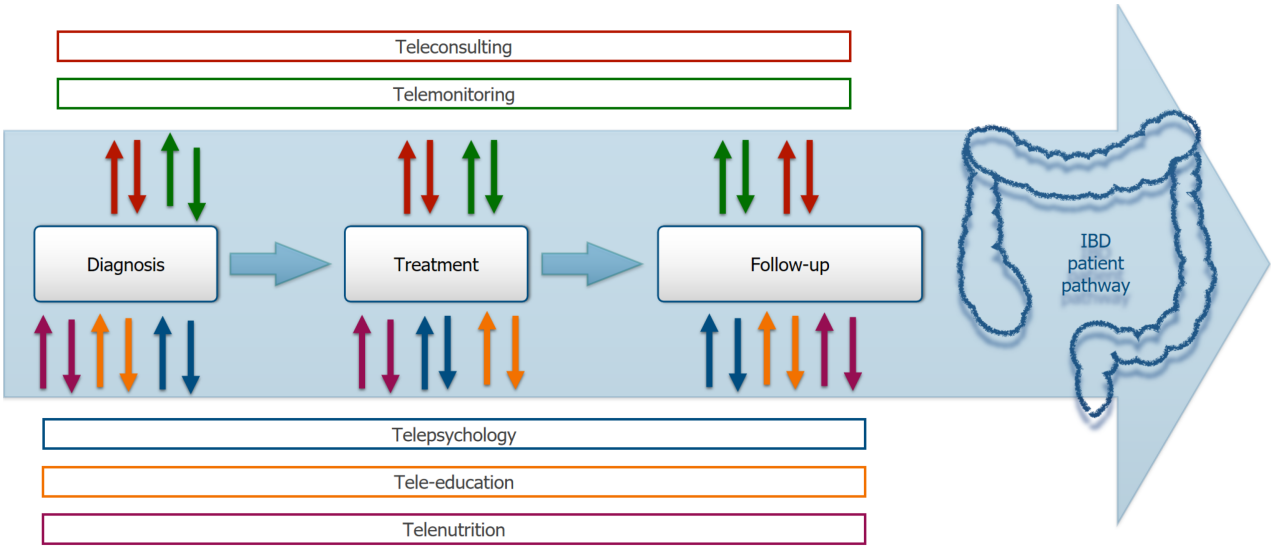
Tele-endoscopy has already been generally proposed for digestive diseases and, in particular, for geographically remote regions[109-112].

However, to the best of our knowledge, no study has been explicitly designed for IBD.

Telepharmacy

Telehealth can also be applied to dispense drugs to patients with IBD through remote contact between the patient and the pharmacist. However, no ad hoc studies have been designed for such applications in the treatment of IBD.

However, there are some initial data on home delivery services set up during the COVID-19 pandemic. For example, in one communication by Ruiz Garcia *et al*[113], they addressed patients with immune-mediated diseases receiving home medication delivery. Among them there were seven IBD patients corresponding to 6.03% of the sample.



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Figure 3 Inflammatory bowel disease patient telemedicine pathway. Based on the current evidence, various telemedicine applications are across the entire inflammatory bowel diseases management, from diagnosis to treatment setting and short-term and long-term monitoring of its effectiveness. Nevertheless, such telehealth applications are bidirectional. That is, they may have the potential to be both active (provided by the physician, psychologist, nurse or nutritionist, *etc.*) and passively requested by the patient from their healthcare managers on needs. IBD: Inflammatory bowel diseases.

CONCLUSION

In conclusion, IBD is a complex disease with many variables in its management, and a disease course that is rarely predictable. Close monitoring of this type of patient is necessary, as they require constant and continuous care, especially when the disease flares. Regular follow-up is also needed to determine whether the therapy is achieving its target. As highlighted by the STRIDE-II consensus, the goal is to ensure a QoL that overlaps that of the general population with the help of medicine. Telemedicine can intervene by meeting the needs of these patients (Figure 3). Many telehealth applications have been studied; however, other applications require considerable attention and new evidence. It is still necessary to study tele-endoscopy, telepharmacy, telesurgery or telerehabilitation in the context of IBD and studies are still awaited. In addition, telehealth has several limitations to consider. That is, it is not clear, under what precise conditions the telehealth medical examination can be superimposed on the in-person examination. It is not yet defined what spectrum of patients may or may not be followed with such remote modalities, although much of the literature addresses patients with mild-to-moderate IBD and not necessarily in advanced therapy. The absence of an objective examination that can be done in attendance is certainly another limitation to consider. Indeed, to achieve these goals, one needs systems that integrate patient-reported symptom data with objective data (*i.e.*, laboratory tests and instrumental examinations) so that the telehealth visit can be as reliable as possible.

Conversely, there is a need to evaluate which patients may be compliant with such systems and can use them fully. There are, however, exciting perspectives and evidence, as outlined in this review on telemonitoring, that make, in any case, telehealth a tool certainly to be considered in the management of chronic patients such as those with IBD. In addition, psychological monitoring tools (considering how much these patients suffer from anxiety-depressive disorders compared to the general population) should be encouraged.

In addition, in many realities, the COVID-19 pandemic was an unexpected and dramatic event, an opportunity for telemedicine systems to be an obligatory and ready solution to solving the medical care shortage. In this context, these efforts, as the pandemic epidemiological situation improves, should not be thwarted and represent an important starting point for the continued implementation of telehealth systems. In addition, the robust research growth that there has been with COVID-19 should similarly not come to a halt. All of these would enable the collection of additional evidence, as much real-life as the result of clinical trials, that could hopefully stimulate precise recommendations from the world's major IBD guidelines.

FOOTNOTES

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Country/Territory of origin: Italy

ORCID number: Antonietta Gerarda Gravina 0000-0001-8049-0115; Raffaele Pellegrino 0000-0001-5074-230X; Giovanna Palladino 0000-0002-7367-4175; Rossella D'Onofrio 0009-0002-4761-0028; Giusi Arboretto 0009-0000-7938-8949; Salvatore Auletta 0009-0008-8565-0120; Giuseppe Imperio 0000-0002-4182-2858; Andrea Ventura 0009-0005-5735-7195; Mario Romeo 0000-0002-2970-9019; Alessandro Federico 0000-0002-0885-0793.

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Utilization of online systems to promote youth participation in research: A methodological study

Marie Salem, Lance Pollack, Alex Zepeda, Kathleen P Tebb

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Marie Salem, Institute for Health Policy Studies, University of California San Francisco, San Francisco, CA 94158, United States

Lance Pollack, Division of Prevention Science, University of California San Francisco, San Francisco, CA 94105, United States

Alex Zepeda, Research, Los Angeles Trust for Children's Health, Los Angeles, CA 90017, United States

Kathleen P Tebb, Department of Pediatrics, University of California San Francisco, San Francisco, CA 94143, United States

Corresponding author: Marie Salem, MPH, Research Scientist, Institute for Health Policy Studies, University of California San Francisco, 490 Illinois St, San Francisco, CA 94158, United States. mariesalemk@gmail.com

Abstract

BACKGROUND

Online surveys can align with youth's increased use of the internet and can be a mechanism for expanding youth participation in research. This is particularly important during the coronavirus disease 2019 (COVID-19) pandemic, when in-person interactions are limited. However, the advantages and drawbacks of online systems used for research need to be carefully considered before utilizing such methodologies.

AIM

To describe and discuss the strengths and limitations of an online system developed to recruit adolescent girls for a sexual health research study and conduct a three-month follow up survey.

METHODS

This methodology paper examines the use of an online system to recruit and follow participants three months after their medical visit to evaluate a mobile sexual and reproductive health application, *Health-E You/Salud iTu*™, for adolescent girls attending school-based health centers (SBHCs) across the United States. SBHC staff gave adolescent girls a web link to an online eligibility and consent survey. Participants were then asked to complete two online surveys (baseline and 3-month follow-up). Surveys, reminders, and incentives to complete

them were distributed through short message service (SMS) text messages. Upon completing each survey, participants were also sent an email with a link to an electronic gift card as a thank-you for their participation. Barriers to implementing this system were discussed with clinicians and staff at each participating SBHC.

RESULTS

This online recruitment and retention system enabled participant recruitment at 26 different SBHCs in seven states across the United States. Between September 2021 and June 2022, 415 adolescent girls were screened using the Qualtrics online survey platform, and 182 were eligible to participate. Of those eligible, 78.0% (n = 142) completed the baseline survey. Participants were racially, geographically, and linguistically diverse. Most of the participants (89.4%) were non-White, and 40.8% spoke Spanish. A total of 62.0% (n = 88) completed the 3-month follow-up survey. Limitations of this system included reliance on internet access (*via* Wi-Fi or cell service), which was not universally available or reliable. In addition, an individual unrelated to the study obtained the survey link, filled out multiple surveys, and received multiple gift cards before the research team discovered and stopped this activity. As a result, additional security protocols were instituted.

CONCLUSION

Online systems for health research can increase the reach and diversity of study participants, reduce costs for research personnel time and travel, allow for continued study operation when in-person visits are limited (such as during the COVID-19 pandemic), and connect youth with research using technology. However, there are challenges and limitations to online systems, which include limited internet access, intermittent internet connection, data security concerns, and the potential for fraudulent users. These challenges should be considered prior to using online systems for research.

Key Words: Online recruitment; Adolescents; Sexual and reproductive health; Mobile data; Methodology paper; Data security

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Core Tip: Online systems for health research have the potential to reach larger and more diverse audiences than traditional in-person recruitment methods. It can also decrease the cost and time necessary to recruit participants in person. This paper provides a case study of the online system developed and used to evaluate *Health-E You/Salud iTu*™, an interactive mobile sexual and reproductive health application (app) for adolescent females used in conjunction with school-based health centers. This study demonstrates the strengths and limitations of online systems used for research.

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INTRODUCTION

Online systems were widely used prior to the coronavirus disease 2019 (COVID-19) pandemic; however, COVID has increased the need for researchers to consider alternative methods to continue their study activities remotely[1,2]. Online systems to recruit and consent participants in research studies can be more time-efficient and reduce costs associated with traditional, in-person approaches[3,4]. More recently, online recruitment efforts for health research have been conducted with targeted web-based strategies on social media platforms[2,5,6]. However, online recruitment can also be useful for clinic-based sampling to evaluate interventions aimed at improving clinical care. In addition, online recruitment methods may be particularly effective for adolescents, given their increased use of mobile technologies, including smartphones, tablets, and laptops[7,8]. There is value beyond recruitment; online systems can be used to automatically send participants links to follow-up surveys, reminders to complete follow-up surveys, and distribute e-gift card incentives[9,10].

The utilization of mobile technologies can extend the geographical reach and promote the participation of diverse adolescent populations in health research. Our study specifically focuses on adolescents aged 13-19 years, as that is the common age of high school students utilizing our participating school-based health centers (SBHCs). It is important to note that there is variation in the definition of adolescents. For instance, the World Health Organization defines adolescence as between ages 10-19[11], while the American Academy of Pediatrics considers adolescence as between ages 11-21[12]. Almost all adolescents in the United States (US) (95%) have access to smartphones, and 45% said that they are "almost constantly" online[8]. This is true for youth from lower socio-economic and diverse racial/ethnic backgrounds. Specifically, 93% of low-income adolescents, 96% of Hispanic, and 91% of Black adolescents have access to a smartphone [8]. Further, among US smartphone owners, young adults, those with no college education, and those with lower income levels are most likely to use their mobile phone as their main source to access the internet[13]. At the same time, studies

show that youth from diverse racial/ethnic, sexual orientation, gender identity, and low-income backgrounds are often underrepresented in research[14-16]. Thus, using these technologies can potentially expand youth participation, increase the diversity of participants[8,17,18], and reach youth from historically under-resourced communities[18]. A recent study comparing virtual recruitment studies to in-person recruitment for medical research found that virtual studies were able to enroll participants from more geographically diverse regions and recruit higher percentages of females[19]. Online recruitment is more cost-effective and time-efficient than in-person recruitment. In-person recruitment involves greater costs associated with travel to clinic study sites and study staff time spent on recruitment activities[5]. Online surveys can also be translated and accessed in multiple languages, further expanding accessibility for non-English speaking participants. It can also include audio features for groups with lower literacy levels[20]. Online surveys are, therefore, an opportunity to expand youth participant reach for populations who have been historically excluded from health research and, in doing so, they can help reduce inequities in research participation related to gender, race, ethnicity, education, location, age, and language spoken.

Using mobile technology for health research data collection can also improve data quality. Online surveys can increase participants' comfort in completing health surveys, especially those on sensitive topics (*e.g.*, sexual health and behaviors)[18]. Research shows that compared to a trained health educator, adolescents are more comfortable disclosing health information on a computer even when they know it will be shared with their clinician[21]. Additionally, online surveys have been shown to reduce social desirability biases compared to interviewer-administered modes[20,22]. In one study that analyzed social desirability for a sexual health survey, respondents who completed the survey over the internet were more likely than those who responded over the telephone to report more than one sexual partner, indicating that online surveys can decrease social desirability bias[22].

Despite the promise of technology in promoting access to research for diverse populations, barriers remain. While adolescents' use of smartphones has increased for all income levels and races/ethnicities, approximately 5% of adolescents still do not have access[8]. The "digital divide" persists in many groups. In particular, those from rural regions[23] and low-income communities[24] across the US have unequal access to reliable internet. Although recent studies show that mobile phone ownership is becoming more evenly distributed among diverse populations[8], divides in internet access exist among school-aged youth of different household incomes[24]. Thus, the "digital divide" has the potential to create barriers to online recruitment efforts and can perpetuate inequalities in health research for some populations.

Online recruitment methods can also create barriers to obtaining informed consent, risk the inclusion of fraudulent users, and hinder participant retention over time. These barriers have been identified in a national study that used online surveys for sexual and gender minority adolescent health research[16]. Obtaining consent online may make it difficult for some participants to fully understand their rights, risks, and benefits of participating in research. Even when the language is simplified, consent can be complicated. When informed consent is done online, there is no research staff present and readily available to answer questions, provide clarification, and ensure the potential participants' understanding[16]. While potential participants can be encouraged to contact research staff with questions, they are not required to do so. To compensate for this limitation and increase comprehension of the consent and study processes, researchers have used videos, consent quizzes, and interactive follow-up methods[16]. Online surveys are also prone to fraudulent or repeat users, especially when incentives are distributed for survey completion[16,25]. For example, an online study on COVID-19's impact on LGBTQ+ populations resulted in 62% fraudulent survey responses due to the infiltration of bots[25]. Actions such as robust built-in data safety measures, requiring the same data point throughout the survey, and avoiding automatic incentive payments when the survey is complete, can decrease fraudulent users. However, implementing these safeguards is time-consuming for researchers and may decrease participation from authorized participants due to increased survey time and potential time delays for incentive distribution after survey completion[25]. In addition, participant retention over time may also be problematic when online recruitment strategies are used due to a lack of personal contact, and participants' early interest in the study may fade over time, especially without a personal connection to the study[26,27].

Despite these technology-related limitations, there are several advantages to online research methodologies. They can increase research during COVID isolation periods[1,2], reduce research-related personnel and travel costs for researchers and participants[5], and improve the feasibility and efficiency of conducting research across multiple geographic regions, thereby increasing diverse populations of youth access to research[8,17,19]. Due to ever-changing technology landscapes, there is a need for additional research on best practices for online survey recruitment, data collection, and participant retention strategies.

There are only a few papers that describe online study processes used in conducting adolescent sexual and reproductive health (SRH) research; however, these papers are not primarily focused on methodologies. These studies include an online human immunodeficiency virus (HIV) prevention study called YouthNet[27], an online adolescent and young adult HIV study called Just/Us[28], and an adolescent SRH study focused on online social media recruitment called SpeakOut[29]. There are a few gaps in this literature that our methodology paper seeks to address. The papers describing YouthNet[27], Just/Us[28], and SpeakOut[29] are focused on online recruitment and enrollment methods, but do not go into detail about survey program, settings, security, monitoring, and tracking. In addition, our study differs from these studies because it: (1) Uses a hybrid approach of recruiting in-person and data collecting online; and (2) engages with a youth advisory board that informed our research methods. Specifically, we engaged youth input to ensure the inclusivity of genders and improve participant retention. This methodology paper aims to address these gaps and expand the literature on online data collection processes for adolescent health research.

MATERIALS AND METHODS

This methodology paper provides a case study of an online system that we developed to evaluate *Health-E You/Salud iTu*™, an interactive, individually tailored mobile SRH application (app) that is available in English and Spanish. The web-based app is a pre-visit tool that provides a brief risk pregnancy-risk assessment, personalized patient education, and contraceptive decision-making support. The app also shares a confidential summary with the clinician, in real-time, prior to the clinician-patient encounter to improve the delivery of patient-centered contraceptive care. In a cluster randomized control trial (RCT) of 18 SBHCs in Los Angeles County, California, with 1360 Latina participants, use of the app increased knowledge, self-efficacy, and use of effective contraception over a 3- and 6-month follow-up[30]. The app is now being disseminated to SBHCs across the US to evaluate the effectiveness of the app on a broader population of adolescents, using a randomized stepped-wedge design and an online recruitment and follow-up system. This study uses a hybrid approach; recruitment occurs in person at SBHCs, and the consent, data collection, and incentive distribution occurs *via* an online system that is detailed in this methodology paper. This study includes adolescents who were sex assigned as female at birth, who have had sexual intercourse in the past three months, not currently pregnant and who are not currently using a long-acting contraceptive (LARC) device. The participants are between the ages of 13-19 year, the age range of most youth served in our participating SBHCs.

Data collection survey process

The eligibility, baseline and follow-up surveys were programmed into the Qualtrics online survey platform[31]. The eligibility survey took less than 5 minutes to complete, and the baseline and 3-month follow-up surveys took approximately 10 minutes. These surveys can be taken on any device (*e.g.*, smartphone, tablet, or computer) connected to the internet.

Clinic staff were asked to provide all adolescents coming into the participating SBHC with a link to the online eligibility survey. Clinic staff distributed the eligibility survey link *via* business cards that had a QR code. In addition, clinics hung posters that included information about the study along with the survey QR code and bit.ly shortened survey link. This link directed adolescents to information about the study, and it then asked about their interest in participating, assessed eligibility, and obtained informed consent.

Consented participants were then asked to provide their cell phone number, which was saved securely in Qualtrics and used to distribute subsequent survey links through SMS text messages[9]. To link the eligibility, baseline, and follow-up surveys and to protect participants' confidentiality, we created a unique participant identifier (ID) that included the participant's first letter of their first name, the first letter of their last name, birth date, and birth month.

After creating a unique ID, participants were immediately texted a link to the baseline survey prior to the visit with their SBHC clinician. This SMS text was generated and distributed through Qualtrics. We also set up the Qualtrics system to distribute texts at 1 and 2 months after baseline to remind participants about when they would receive the link for the 3-month follow-up survey with the goal of increasing retention rates. In addition, 3 months after baseline, participants received an SMS text with the link to the 3-month follow-up survey. Participants received SMS text reminders to complete the 3-month survey beginning 24 hours after receiving the link and every other week for up to 2 months (for a maximum of three text reminders and two email reminders) as part of our retention efforts.

The National School-Based Health Alliance Youth Advisory Board (YAB) informed the language on the SMS texts and schedule of the SMS reminders to maximize recruitment and retention. The YAB recommended that the name of the principal investigator be included to increase the "friendliness" of the text and decrease the risk of it appearing as spam. They reviewed the language of the messages to ensure that they were gender-inclusive, reduced the length of the text messages, and provided language about the incentives for completing the survey. They also provided input on the look of the cards and posters used at the SBHCs to promote the study.

Electronic gift card incentive system

Participant incentives were also distributed through an online system. Qualtrics was used in conjunction with an electronic gift card (e-gift card) system. For this study, we used Rewards Genius, operated by Tango Card[32]. The Qualtrics/Tango integration was included with our University's Qualtrics license and allowed for the automatic distribution of digital rewards (e-gift cards) to survey respondents. The Rewards Genius system allowed us to select the monetary amount of the gift card for each specific survey, limit the maximum amount of gift cards distributed, and included a setting to prevent the distribution of multiple gift cards to the same email for the same survey. Rewards Genius has a self-serve online portal to track how many incentives have been sent and monitor the incentive budget. At the end of each survey, the participant was asked how they would like to receive their gift card. If they provided their email, the participant immediately received an email from Tango with a link to redeem a gift card of their choice. If a participant did not have (or did not provide) an email, they could request that their gift card be texted to them. For the SMS option, gift cards were sent manually (as there was no automated option available in this system). To do this, the research assistant downloaded survey data from Qualtrics every other week and used Stata to export the participants' cell phone numbers, without a corresponding email, into an Excel sheet. Research staff manually texted each of these participants with a link to a gift card.

Survey settings

Qualtrics has a variety of setting options that are important to consider for each survey. In this study, Qualtrics was set to record incomplete surveys for partial data after 1 week for the eligibility survey and 1 month for the baseline and 3-month follow-up surveys. The eligibility survey was open for 1 week to decrease the chance of an individual using the

QR codes multiple times. The 3-month follow-up survey remained open for 1 month after the initial completion date to increase participant response rates. Each survey included a back button for participants to change or review their responses, skip logic to route participants to different questions based on their prior responses, and a Qualtrics setting to ensure that email and phone numbers were entered correctly.

Data storage and security

Data was securely stored in the Qualtrics database. Qualtrics is General Data Protection Regulation (GDPR) and California Consumer Privacy Act (CCPA) compliant and provides technology for users to be compliant as well[33]. As mentioned previously, to further protect participants' confidentiality, data from the eligibility survey were stored separately from the baseline and follow-up surveys. The baseline and follow-up surveys included the participant's unique ID, so there was no way to identify an individual with their survey responses, if in a rare event, the back-end database was hacked. Rewards Genius and Tango Card also protect data and privacy through GDPR and the CCPA in addition to multi-factor authentication[32]. Only authorized research staff had access to these systems. We also set up a code within Qualtrics that includes reCAPTCHA (Completely Automated Public Turing Test to Tell Computers and Humans Apart) data to identify bots and relevant ID data to prevent duplicate responses, fraudulent users, and the distribution of multiple gift cards to any individual user. The security system in Qualtrics was vital because our online surveys were attached to incentive gift card distribution. Participants or hackers may be motivated to complete the surveys multiple times with inaccurate responses to obtain e-gift cards[33].

Data monitoring

Monitoring participant enrollment, follow-up survey completion, and gift card distribution were critical to the integrity of this study. Data, including the participant variables included in the unique ID and participant contact information from the eligibility, baseline, and 3-mo surveys, were downloaded from Qualtrics by SBHC in each state. The participant's unique ID was used to match the baseline and follow-up surveys using Stata programming. The Stata code identified potential duplicates, participant identification data errors, and participants without emails who need manual (SMS) gift card incentive deliveries. Fraudulent and repeat users were identified during this data monitoring process through duplicate unique IDs, emails, or phone numbers. The code also produced Excel files of unmatched surveys, including participants who were eligible but did not complete a baseline survey and/or participants who completed the baseline but not the 3-month follow-up survey. This monitoring system allowed us to manually send unmatched participants an SMS text reminder with the survey link. As part of data monitoring efforts, enrollment and 3-month survey completion rates were provided to each participating SBHC on a monthly basis. The investigators discussed this data with clinicians and staff champions from each site, along with implementation successes and challenges.

We worked with SBHCs in diverse locations that largely serve youth who are underserved by the broader health care system. To increase the representation and diversity in our sampling, we asked SBHC staff to provide all adolescents coming for care the opportunity to participate in the study and use the app (when in intervention mode). In addition, we used data monitoring to ensure that the online sampling was representative of youth at the SBHCs. For each school year, we compared the demographics of the survey responses with the retention rates. While we encouraged the distribution to all adolescents and had an incentive system to encourage participation, our sample is one of convenience and relied on the youth's willingness to participate.

The statistical methods of this study were reviewed by Lance Pollack, PhD at the University of California San Francisco.

Ethical approval

Informed consent by all participants were obtained through an online consent process. The research protocols and study were approved by ethics review boards at the University of San Francisco.

RESULTS

Participant enrollment and retention

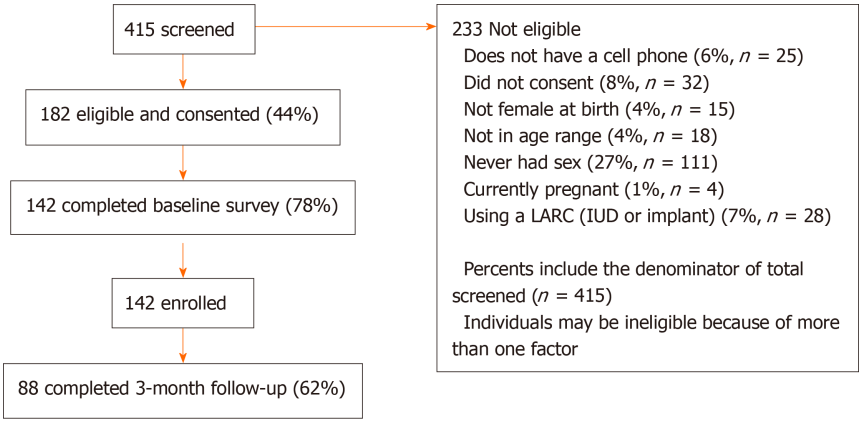
In the academic year between September 2021 and June 2022, 26 SBHCs across seven states: California, Illinois, Massachusetts, Michigan, Minnesota, New York, and Texas agreed to participate in this study. A total of 415 adolescents were screened to determine eligibility. The screening rate was based on participants who at least clicked on the link that the SBHC staff provided them. Of these, 43.9% (n = 182) were eligible based on study inclusion criteria. There were no significant differences between ineligible and eligible participants when comparing age, race/ethnicity, and language spoken at home. Of the 182 eligible participants, there was a 78.0% (n = 142) retention rate of those who were enrolled and completed the baseline survey (Figure 1). Of the enrolled participants, all were female sex assigned at birth (per inclusion criteria), 95.1% (n = 135) were identified as female, 2.8% (n = 4) identified as non-binary, 1.4% (n = 2) as male or transgender male, and 0.7% (n = 1) as gender fluid (Table 1). The mean age was 16.7 (SD +/- 1.1) years. Nearly half (49.3%) of the participants identified as Hispanic/Latin, 15.5% Black/African American, 12.7% Asian, 10.6% White/Caucasian, and 12% multi-racial/ethnic. Many (40.8%) spoke Spanish with their family, either solely or in addition to English.

Of those who were enrolled in the study, there was a 62.0% (n = 88) retention rate of those who completed the 3-month follow-up survey. There were no significant differences in age, race/ethnicity, and languages spoken at home between

Table 1 Descriptive statistics of participants at baseline[a,b,c]

Demographics	*n* (%)
Gender	
Female	135 (95.1)
Male	1 (0.7)
Transgender male	1 (0.7)
Non-binary	4 (2.8)
Gender fluid	1 (0.7)
Sex assigned at birth	
Female	142 (100)
Age group, mean +/- SD	16.7 +/- 1.1
Race/ethnicity	
Asian	18 (12.7)
Black/African/African Amer	22 (15.5)
Hispanic/Latinx/o/a	70 (49.3)
White/Caucasian	15 (10.6)
Multi-racial/ethnic	17 (12)
Speaks Spanish with family	58 (40.8)

[a]*N* = 142 unless otherwise noted.
[b]Outcomes are *n* (%) unless otherwise noted.
[c]Percentages may not add up to 100 due to rounding.



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Figure 1 Participant flow diagram.

those who completed the 3-mo follow-up and those who did not (Table 2). Among the 3-month follow-up survey participants, 95.5% were identified as female with a mean age of 16.8 years (SD +/- 1.04). Nearly half (46.6%) identified as Hispanic/Latin, 11.4% Black/African American, 14.8% Asian, 12.5% White/Caucasian, and 14.8% reported being multi-racial/ethnic.

Challenges

There were a few challenges that resulted with this online data system. As part of ongoing data monitoring efforts, we provided SBHC clinicians and staff monthly data and discussed implementation barriers. Some staff reported that it was difficult to remember to distribute the link because they had other responsibilities, were short-staffed, or they forgot when the adolescent was being seen for a non-reproductive health visit. A total of 21 out of the 26 SBHCs reported being short-staffed or overworked due to ongoing challenges with COVID-19 that increased rates of illness for staff and their family members, exacerbated youth mental health issues, and contributed to burn-out. Additionally, some clinicians and

Table 2 Descriptive statistics of participants at 3-mo follow-up[a,b,c]

Demographics	*n* (%)
Gender	
Female	84 (95.5)
Transgender male	1 (1.1)
Non-binary	3 (3.4)
Sex assigned at birth	
Female	88 (100)
Age group, mean +/- SD	16.8 +/- 1.04
Race/ethnicity	
Asian	13 (14.8)
Black/African/African Amer	10 (11.4)
Hispanic/Latinx/o/a	41 (46.6)
White/Caucasian	11 (12.5)
Multi-racial/ethnic	13 (14.8)
Speaks Spanish with family	34 (38.6)

[a]*N* = 88 unless otherwise noted.
[b]Outcomes are *n* (%) unless otherwise noted.
[c]Percentages may not add up to 100 due to rounding.

staff reported that internet access and connectivity problems were barriers to youth accessing and completing the surveys. There were internet connectivity issues for cellular data at five SBHCs, limiting access for youth to use their phones to complete the surveys. The high school affiliated with two SBHCs, did not allow students to use their cell phones on campus so youth could not access the internet until these sites were able to connect tablets to their clinic Wi-Fi, which involved setting up a secure guest wifi network. As a result, not all potential participants received the eligibility survey link.

Despite protections against duplicate and fraudulent users, there were some duplicate users that we identified and removed, and one individual, not related to the study, hacked the system and obtained multiple e-gift cards. Duplicate users came from SBHCs that provided adolescents with iPads to access the online eligibility survey. Since multiple youth were using the same device, a generic link had to be used to access the online system. Thus, it was not possible to set up Qualtrics to limit the number of times that a survey was completed for respondents using the same device. Duplicates were removed as part of the data monitoring and cleaning process. Additionally, one SBHC posted the survey link on their social media website. An individual, not involved with this SBHC, obtained the survey link to access the survey, identified the study eligibility criteria, and generated multiple, fictitious participant contacts to complete multiple baseline surveys and obtain incentives. The hacker used the system over the weekend and by Monday, when study staff returned to work and discovered the problem through routine data monitoring, the hacker completed 668 surveys and obtained $6680 in gift cards. This was the only time in which this issue occurred. All SBHCs were advised to distribute the link only to individual patients coming to their clinic and avoid posting the link on social media or other clinic websites. There was no breach of any participant data during this incident.

DISCUSSION

This methodological paper provides a case study of the online system used to evaluate the *Health-E You/Salud iTu*™ app. We found that this system enabled us to recruit and retain a diverse study population without having to deploy study personnel to the 26 SBHC sites across seven states. We found no statistically significant differences between eligible and non-eligible participants based on age, race/ethnicity, and language spoken at home. There were also no significant differences between participants who completed the baseline and participants who completed the 3-month follow-up survey based on age, race/ethnicity, and language spoken at home.

Strengths of the online system

A major strength of this online research system was its ability to reach a diverse group of youth at multiple SBHCs across the nation including Northern and Southern California, Illinois, Massachusetts, Michigan, Minnesota, New York, and Texas. In addition to geographic diversity, this study recruited a racially diverse sample size with almost half coming

from Hispanic/Latin backgrounds and the balance comprising relatively equal proportions of Black, Asian, White, and multi-racial/ethnic participants. Additionally, almost half respondents reported that they were Spanish speakers. The online system allowed participants to participate in their preferred language English or Spanish and they could toggle between languages throughout the process. Increasing diversity of participants' region, race, and language spoken is a vital aspect to increasing inclusivity and representation of underrepresented populations in health research.

Online research systems can reduce costs and increase efficiency for researchers. Online research systems allow for increased organization, centrally located documents and materials, and an efficient tracking system to save time for researchers. The study recruitment and surveys were set up online with automatic survey distributions and reminders using Qualtrics settings, which allowed our research staff to have more time to track and communicate with participants individually, meet with clinics more frequently, create and meet with the youth council, and make adaptations to improve the study. Online surveys saved costs on transportation, survey materials, and data collection staff. In this study, these aspects would have been costly due to the number of participating SBHCs across multiple states in the US. However, it should be noted, that there can be research costs when a study uses paid adds to recruit *via* social media or through paid online survey platforms[5] (neither of these were used in this study). Our hybrid approach that included initial in-person contact (by SBHC staff) followed by online data collection and monitoring, cost less than other online studies[27,29,35]. An online HIV study spent $13,000 on banner advertising[27] and a smoking-cessation study spent $172.76 per participant on social media[35]. In contrast, our study spent approximately $32.77/clinic on promotional materials for direct study recruitment.

Online surveys are also an extremely effective way to increase the accuracy of survey responses from adolescent participants as well as their comfort responding to sensitive questions about their SRH. In previous SRH studies, adolescents have reported concerns about clinician judgment, power differential, and a lack of confidentiality[34]. Research also shows that adolescents feel more comfortable answering SRH questions online compared to clinician or researcher interview[21]. In addition, while not examined in the current study, prior research found that social desirability bias occurred when research was conducted in person or on a phone call rather than online[22].

Limitations of the online process

Despite the advantages of online systems, such as those used in the current study, there are important challenges to consider. While asking clinic staff to distribute the link did not seem like it would be burdensome, staff commonly forgot to do so. Clinics reported being short-staffed, overworked, and were focused on other pressing priorities at their clinic and/or with their patients – most of which were related to ongoing challenges associated with the COVID-19 pandemic.

Consistent with prior research[16,25], our study found that online recruitment and follow-up surveys can increase the possibility of duplicate survey responses and invalid survey participants or hackers. By automatically linking online surveys to gift card incentive distributions (participants receive an e-gift card directly after survey completion), hackers can take advantage of incentive systems to target the survey through code that searches the internet for surveys linked to gift cards. To prevent unsolicited participants from finding online surveys, survey links should not be posted online on public platforms. However, restricting access to survey links can also limit broader recruitment through online platforms such as social media sites. To maximize reach and limit fraud, gift cards could be manually distributed *via* text or email after each survey is verified and/or researchers can consider validating the authenticity of the participant through follow-up phone calls prior to study enrollment. However, both of these methods can increase the burden for research staff and create delays in the distribution of participants' gift cards. To limit these duplicate and fraudulent user issues, we implemented robust security systems in Qualtrics (described in the methods section); however, there was still a possibility for error of fraudulent or duplicate users in these security systems. Researchers as well as potential participants need to better understand the risks of online research, such as fraudulent users, duplicates users, and potential data security breaches, especially in the context of rapidly evolving computer-based and online technologies.

While the retention rates in this study are comparable to other online studies focused on SRH, there is a need to improve retention rates in online studies. The recruitment and 3-month retention rates in this study were higher than those of the original cluster RCT (78% *vs* 57% for recruitment rates and 3-month retention rates were 62% *vs* 50%, respectively)[30]. These improvements in the current study may be due to lessons learned from the original trial, additional input from the YAB, and the expanded diversity of the study population. One advantage of this study's online system, compared to the original RCT, was the use of automatic reminders for surveys through Qualtrics. Our retention rates were also greater than that of another online HIV prevention study, which had a 53% retention rate at the 2-month follow-up[27]. This HIV study relied heavily on online recruitment through banner advertisements, which is advantageous for reaching a larger and more diverse study sample; however, using this approach resulted in approximately 20% of potentially fraudulent participants. Our study was able to overcome this issue because, by its very nature, the intervention, use of the *Health-E You* app, is designed to be used in conjunction with a health care visit. While data collection was done *via* our online system, SBHC clinic staff provided the opportunity to use the eligibility link to begin the online system with real patients. In addition, we verified participants by requiring phone numbers to receive future surveys and matched across surveys using the match ID and email addresses. In another online HIV study with adolescents and young adults, retention rates were 69% at the 2 month follow-up and 50% at the 6 month follow-up[28]. While this is more comparable to our study, these rates are lower than those found in other SRH studies conducted in person[28]. In contrast, the retention rates in the current study are lower than those found in a study that recruited adolescents *via* social media[29] whose retention rates ranged from 71% (in the control group) to 79% (intervention). This is likely due to the fact that once deemed eligible *via* the online system, a research assistant conducted a follow-up phone call to verify eligibility and randomize participants to the intervention or control condition. This approach was not possible in our study, since participants were recruited in clinic and the baseline survey had to be completed prior to seeing their clinician. Recruiting *via* social media is advantageous for recruiting a greater number of individuals over a

shorter time period as was demonstrated in the SpeakOut study[29]. In this study, high retention rates can be attributed to the work of research assistants who called each participant to verify their eligibility. This verification process improved the integrity of the data; however, this is a time-consuming and costly approach (in terms of staff time). When we compared our study's retention rates with a smoking cessation study conducted on line, our retention rates were higher [35]. That study had an overall 52% 3-month follow-up retention rate and did not find any significant differences in retention rates between the online methods (including social media, ads, and standard media) *vs* the traditional recruitment methods that they used[35]. Despite these variations in retention rates, some research suggests that the overwhelming majority (82%) of young adults prefer online surveys over mail-in surveys and this preference was greater not only for youth of younger ages, but also for those from higher socio-economic backgrounds[36]. On going efforts to improve retention rates for all participants are needed.

To improve retention rates, researchers have used immediate incentives, continual contact information collection, and consistent reminders[28,29,35,36]. Our study also used these strategies and resulted in comparable or slightly better retention rates than those found in other studies using similar online methods. Thus, it is important to identify and further investigate additional approaches to recruit and increase participant retention when using online methodologies. While the use of online systems for research can increase the reach and diversity of study populations, relatively low retention rates can limit the generalizability of study findings.

Researchers using online systems also need to make special efforts to include participants who do not have internet access. Online surveys require internet access through cellular data, Wi-Fi connection, or ethernet cable connection and this can prevent individuals without such access from participating in research. They may not have cell phones that can access the internet or may have limited data plans with their smartphones. Others may not have household internet access, especially if they live in rural and other underserved areas. Data from the National Center for Education Statistics found that the percentage of households (with youth aged 3 to 18 years old) with home internet access was highest among those who were Asian (99%) and White (97%), and lowest among those who were American Indian/Alaska Native (83%), Pacific Islander (90%), and Black (91%)[37]. Reasons for lack of access included "did not need it/not interested" (50%) and "internet too expensive" (26%)[37]. Additionally, rural US residents also experience lower access to home internet (72%) compared to their suburban (79%) and urban (77%) counterparts[23]. In 2021, the United Nations adopted a resolution on the internet as a human right and encouraged countries to adopt "national internet-related public policies that have at their core the objective of universal access"[38]. However, the US lacks a national policy guaranteeing universal access and internet access disparities remain. Because online survey research systems are dependent on internet access, it is important for future research to explore ways to recruit and retain participants with more equitable rates rather than excluding populations with limited access. This may require hybrid approaches that use a combination of internet and in-person recruitment methods and partnering with community-based organizations to reach and include groups underrepresented in research.

Another limitation of online-only research studies is the lack of in-person interaction. Although the online system can decrease the time that clinics are required to dedicate to the study, it also limits the interpersonal connection that researchers make while in the clinics. To compensate for this limitation, our research team met with the clinic staff every few months *via* Zoom; however, a few clinics were unresponsive over email. In-person interaction could potentially increase clinic staff engagement in the study. The lack of in-person contact with participants, may also contribute to lower follow-up rates as they may feel less of a connection to the researchers and with the study.

Lastly, our study is limited because its findings are not generalizable to all adolescents. Our study participants are female at birth, attend SBHCs, are between the ages of 13-19 years, and not currently pregnant or using a LARC device. However, our online methods are still relevant to the overall literature on adolescent health.

Future implications

This case study of an online system for health research has implications for future research. Ensuring the security of participants' data is a top priority as is maintaining the authenticity of participants and data quality. Although there is increasing information available to the public about data security in settings such as jobs, browsing the internet[39], and social media[40], there is a lack of research describing how to ensure data security in research settings. As shown in our study, added measures are critical to safeguard the distribution of electronic gift cards. Researchers need to be fully aware of these risks and vigilant in protecting and monitoring data security[41]. However, health researchers are often not trained in or aware of all of the details involved with data security and need to partner with experts within and/or outside of their institution to ensure that the best possible security protections are in place, such as separately storing data, securing public Wi-Fi networks, and using data leak prevention and protection systems[42,43]. In this study, we worked closely with Qualtrics representatives and our UCSF Salesforce team. With ever evolving cyber-attacks, new technologies emerge to address this growing threat such as data storage programs and security protected online files[44]. It is vital for future studies to specifically address ways to maximize outreach and recruitment while ensuring the authenticity of the participants and their responses. This involves exploring online and social media recruitment but instituting robust security measures to verify participants such as contact information validation and mobile device authentication [25].

Although this study displayed ways that online systems can reach more racially, geographically, and linguistically (language) diverse populations, there are still disparities in online access through limited Wi-Fi and/or cell service[24]. It is important for studies to continue to identify strategies that include diverse populations through online systems and consider hybrid approaches when online inclusion is not possible. This includes conducting research activities in person with populations that have limited device or internet access. Lastly, there is a need to research the online system limitations related to engagement and the connection between the researcher and participant or clinic staff. It would be helpful for future studies to analyze the extent to which participant engagement is lost in online studies as opposed to in-

person studies. As these limitations are addressed, online systems can be a powerful strategy for increasing the reach and diversity of populations included in health research.

CONCLUSION

Overall, this methodological paper displays the importance of online systems for youth participation in health research and provides examples of methods used to maximize efficiency of these systems. This case study of the *Health-E You/Salud iTu*™ app discusses the use of online systems that helped recruit youth *via* their mobile devices, distribute surveys, monitor participants, and deliver reminders. These systems improved the geographic reach and inclusion of diverse youth participation. Additionally, in an era of COVID-19 remote work, increased use of telehealth, and youth's use of technology, online systems are crucial for health research. Future studies should study how to leverage technology to further improve reach, diversity, and inclusion of underrepresented groups in research.

ARTICLE HIGHLIGHTS

Research background

Online surveys can align with youth's increased use of the internet and can be a mechanism for expanding youth participation in research. The utilization of mobile technologies can extend the geographical reach and promote the participation of diverse adolescent populations in health research. Using mobile technology for health research data collection can also improve data quality. However, the advantages and drawbacks of online systems used for research need to be carefully considered before utilizing such methodologies.

Research motivation

There are few methodology papers that describe online study processes in the same field as our study, adolescent sexual and reproductive health (SRH). These studies that describe online methodologies include an online human immunodeficiency virus (HIV) prevention study called YouthNet, an online adolescent and young adult human immunodeficiency virus study called Just/Us, and an adolescent SRH study focused on online social media recruitment called SpeakOut. However, there is a lack of research on how to specifically involve adolescents in research and the methods used to ensure diversity, keep the adolescents retained, and maintain data security.

Research objectives

The purpose of this study is to describe and discuss the strengths and limitations of an online system developed to recruit adolescent girls for a sexual health research study and follow them for 3 months. It aims to address the gap of methodology papers and expand the literature on online data collection process for adolescent health research.

Research methods

This methodology paper examines the use of an online system to recruit and follow participants to evaluate a mobile SRH application, *Health-E You/Salud iTu*™, for adolescent females attending school-based health centers (SBHCs) across the US. The paper goes into detail regarding the following methodologies for our online study: Data collection and survey processes, the electronic gift card incentive system, survey settings, data storage and security, and data monitoring.

Research results

This online recruitment and retention system enabled participant recruitment at 26 different SBHCs in seven states across the United States. Between September 2021 and June 2022, 415 adolescent girls were screened using the Qualtrics online survey platform, and 182 were eligible to participate. Participants were racially, geographically, and linguistically diverse; most of the participants (89.4%) were non-White, and 40.8% spoke Spanish. Limitations of this system included reliance on internet access (*via* Wi-Fi or cell service), which was not universally available or reliable, and some issues individuals outside the study discovering the survey link and completing multiple surveys.

Research conclusions

Online systems for health research can increase the reach and diversity of study participants, reduce costs for research personnel time and travel, allow for continued study operation when in-person visits are limited (such as during the coronavirus disease 2019 pandemic), and connect youth with research using technology. The methods detailed using online surveys, online gift card distribution, and online data monitoring and tracking are new and add to the lack of methodology papers. However, there are challenges and limitations to online systems, which include limited internet access, intermittent internet connection, data security concerns, and the potential for fraudulent users. These challenges should be considered prior to using online systems for research.

Research perspectives

This case study of an online system for health research has implications for future research. Ensuring the security of participants' data is a top priority as is maintaining the authenticity of participants and data quality and as shown in our

study, added measures are critical to safeguard the distribution of electronic gift cards. It is important for studies to continue to identify strategies that include diverse populations through online systems and consider hybrid approaches when online inclusion is not possible. Lastly, there is a need to research the online system limitations related to engagement and the connection between the researcher and participant or clinic staff.

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FOOTNOTES

Author contributions: Salem M and Tebb KP performed the research; Tebb KP, and Pollack L designed the research study; Pollack L analyzed the data; Salem M, Tebb KP, and Zepeda A wrote the manuscript; all authors have read and approved the final manuscript.

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Country/Territory of origin: United States

ORCID number: Marie Salem 0000-0002-0431-2680.

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Basic Study

Comprehensive analysis of cell-extracellular matrix protein Ras suppressor-1 in function and prognosis of gastrointestinal cancers

Ya Xu, Yan-Yu Hou, Zheng Wu, Ze-Xuan Fang, Hua-Tao Wu, Jing Liu

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Ya Xu, Department of Radiation Oncology, Shenshan Medical Center, Memorial Hospital of Sun Yat-sen University, Shanwei 516600, Guangdong Province, China

Yan-Yu Hou, Zheng Wu, Ze-Xuan Fang, Jing Liu, Guangdong Provincial Key Laboratory for Diagnosis and Treatment of Breast Cancer, Cancer Hospital of Shantou University Medical College, Shantou 515041, Guangdong Province, China

Hua-Tao Wu, Department of General Surgery, The First Affiliated Hospital of Shantou University Medical College, Shantou 515041, Guangdong Province, China

Corresponding author: Jing Liu, MD, PhD, Academic Research, Associate Professor, Research Scientist, Senior Scientist, Guangdong Provincial Key Laboratory for Diagnosis and Treatment of Breast Cancer, Cancer Hospital of Shantou University Medical College, No. 22 Xinling Road, Shantou 515041, Guangdong Province, China. jliu12@stu.edu.cn

Abstract

BACKGROUND

Ras suppressor 1 (RSU1), a highly conserved protein, plays an important role in actin cytoskeleton remodeling and cell-extracellular matrix adhesion. Aberration of RSU1 activity can cause changes in cell adhesion and migration, thereby enhancing tumor proliferation and metastasis. However, the correlation between RSU1 and gastrointestinal cancers (GICs), as well as its prognostic role related to tumor-infiltrating immune cells (TIICs) remains unclear.

AIM

To shows RSU1 plays a potential promoting role in facilitating tumor immune escape in GIC.

METHODS

Differential expression of RSU1 in different tumors and their corresponding normal tissues was evaluated by exploring the Gene Expression Profiling Interactive Analysis (GEPIA) dataset. The correlation between RSU1 expression and prognosis of GIC cancer patients was evaluated by Kaplan-Meier plotter. Then, RSU1-correlated genes were screened and functionally characterized *via* enrichment analysis. The correlation between RSU1 and TIICs was further characterized using the Tumor Immune Estimation Resource (TIMER). In addition, the correlation between RSU1 and immune cell surface molecules was also analyzed by TIMER.

RESULTS
High RSU1 expression was associated with poor overall survival of gastric cancer patients, exhibiting a hazard ratio (HR) = 1.36, first progression HR = 1.53, and post progression survival HR = 1.6. Specifically, high RSU1 Levels were associated with prognosis of gastric cancer in females, T4 and N3 stages, and Her-2-negative subtypes. Regarding immune-infiltrating cells, RSU1 expression level was positively correlated with infiltration of CD4+ T cells, macrophages, neutrophils, and dendritic cells (DCs) in colorectal adenocarcinoma and stomach adenocarcinoma. RSU1 expression was also predicted to be strongly correlated with immune marker sets in M2 macrophage, DCs and T cell exhaustion in GICs.

CONCLUSION
In gastrointestinal cancers, RSU1 is increased in tumor tissues, and predicts poor survival of patients. Increased RSU1 may be involved in promoting macrophage polarization, DC infiltration, and T cell exhaustion, inducing tumor immune escape and the development of tumors in GICs. We suggest that RSU1 is a promising prognostic biomarker reflecting immune infiltration level of GICs, as well as a potential therapeutic target for precision treatment through improving the immune response.

Key Words: Ras suppressor 1; Gastrointestinal cancer; Immune infiltration; Prognosis; Actin cytoskeleton remodeling

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Core Tip: Ras suppressor 1 (RSU1), is a highly conserved protein involved in actin cytoskeleton remodeling and cell-extracellular matrix adhesion. The current study provides a comprehensive analysis of RSU1 in gastrointestinal cancer and shows its potential promoting role in facilitating tumor immune escape.

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INTRODUCTION

The International Agency for Research on Cancer has released new data on the global burden of cancer for 2020. China has the world's highest number of new cases and deaths. There were 4.57 million new cancer cases in China in 2020, the top four being lung, colorectal, stomach and breast cancers. At the same time, the top five cancer deaths in China in 2020 were recognized as lung, liver, stomach, esophageal and colorectal cancers[1], with the incidence and mortality of gastrointestinal cancers (GICs) showing a clearly increasing pattern[2]. Currently, the prognosis of GIC patients has been slowly improving with the continuous progress of therapeutic strategies and extensive application of surgery, chemotherapy, biological targeted therapy, immunotherapy and other therapeutic methods[3]. However, poor clinical therapeutic effects remain for a subpopulation of GIC patients, showing the need for more specific and sensitive therapeutic targets for GIC patients. Development of new targeted drugs is a promising method in the treatment of GICs.

On the other hand, immune checkpoint inhibitors have emerged as promising antitumor drugs for multiple tumor types, either as single agents or in combination with chemotherapy[4]. For example, the KEYNOTE-062 and CheckMate-649 phase III clinical trials achieved positive results for immune checkpoint inhibitors in advanced first-line treatment of patients with gastric cancers (GCs)[5,6]. Therefore, clinical guidelines in many countries recommend immunotherapy combined with chemotherapy as the first-line treatment for metastatic GCs[7]. However, it is not clear how to select GIC patients who will benefit most from checkpoint inhibitors, necessitating further investigation for novel biomarkers to predict outcomes and immunotherapeutic response.

Ras suppressor 1 (RSU1) was originally found to suppress RAS-dependent oncogenic transformation, and localizes to human chromosome 10p13[8]. Later, it was found that RSU1 binds with high affinity, *via* its LRR domain, to the LIM5 domain of PINCH1, thereby forming an IPPR complex with ILK-PINCH-Parvin to regulate cell adhesion at sites of focal adhesion[9,10], and participate in physiological and pathological processes of local adhesion and tumor metastasis[11,12]. RSU1 has long been associated with cancers, but its expression in various cancer types or its role in metastasis has been unclear. It has been suggested that RSU1 is involved in the regulation of migration and invasion of breast cancer, liver cancer and brain cancer cells, and has the function of enhancing metastasis in a cell type-dependent manner[11,13-15]. RSU1 could also serve as a therapeutic anti-metastatic target in liver and breast cancer[16]. To evaluate the potential role of RSU1 in GIC, the correlation of RSU1 expression with prognosis in GIC patients with various clinicopathological factors was investigated, as well as the function of RSU1 in the occurrence of GIC and the correlation between RSU1 and immune infiltration.

MATERIALS AND METHODS

The expression pattern of RSU1 in different types of cancers

This platform includes RNA sequencing data from 9736 tumor tissues and 8587 normal tissues from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases. The main functions of GEPIA (http://gepia.cancer-pku.cn/) include gene expression analysis, gene correlation analysis, survival analysis, similar gene prediction, and dimension reduction analysis. The expression of RSU1 in tumors and normal tissues was analyzed *via* GEPIA.

The prognostic value of RSU1 in malignancies

Kaplan-Meier plotter (http://kmplot.com/analysis/) contains 10461 cancer samples for assessing the impact of 54675 genes on survival. Patients with cancers were divided into two groups (high- *vs* low-expression) to analyze the poorer overall survival (OS), poorer first progression (FP), progression free survival (PFS), and poorer post-progression survival (PPS) by means of HRs, 95% confidence intervals and log-rank *P* values. To predict the prognostic values of RSU1, GEO datasets were analyzed through the PrognoScan databases (http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html) based on Cox *P* values.

The signaling pathway associated with RSU1

DAVID (https://david.ncifcrf.gov/conversion.jsp) is a biological information database that integrates biological data, using analytical tools, to provide systematic and comprehensive annotation of biological functions for large-scale gene or protein lists, and extract biological information of interesting genes. RSU1-related genes were analyzed, regarding the top 10 highly enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) items.

The immune infiltration regulated by RSU1 in GICs

TIMER 2.0 (http://timer.cistrome.org) uses state-of-the-art algorithms to provide more reliable estimates of immune infiltration levels for TCGA or user-provided tumor profiles. TIMER 2.0 provides three modules, Immune Association, Cancer Exploration and Immune Estimation, for investigating associations between immune infiltration and genetic or clinical characteristics. TIMER was applied to analyze the expression of RSU1 in GIC patients and its relationship with 6 types of infiltrating immune cells (B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and DCs). The abundance of T cells, neutrophils, macrophages, and DCs, as well as tumor purity are important factors affecting immune infiltration in tumor samples analyzed by genomics.

ESTIMATE was conducted in the open-source R software to analyze the gene expression profiling of GICs for StromaScore, ImmuneScore and ESTIMATEScore. The datasets were downloaded from the GEO database, GSE17536, which had 177 patients with colorectal cancer (CRC), and GSE62254, which had 300 patients with GC. All patients were divided into two groups, high RSU1 *vs* low RSU1 based on the median RSU1 expression level.

Statistical analysis

The survival results from Kaplan-Meier plotter and GEPIA2 are displayed with HRs and *P* or Cox *P* values based on the log-rank test. Spearman correlation and statistical significance were used to evaluate the correlation of gene expression with immune biomarkers in GICs. Differences with $P < 0.05$ were considered significant.

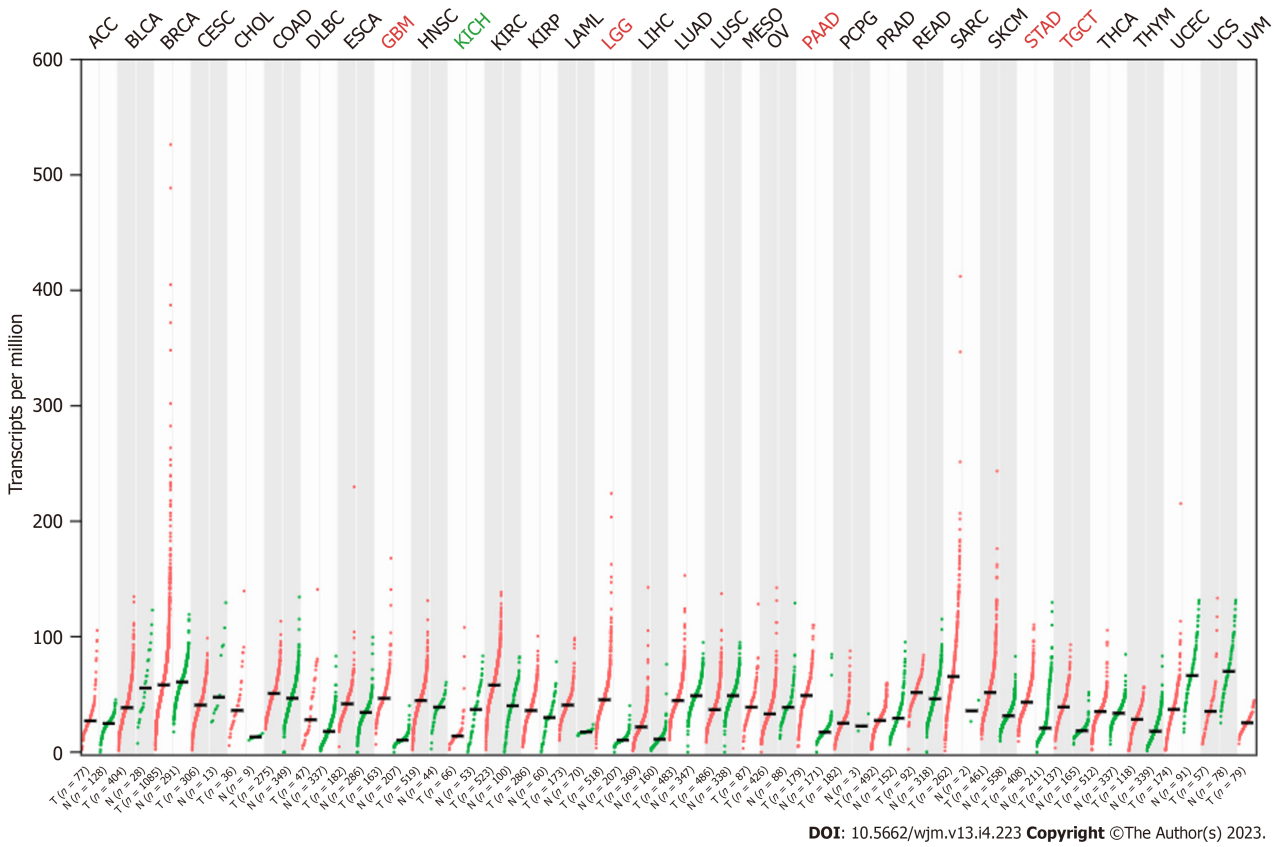
RESULTS

Expression levels of RSU1 mRNA in different types of human cancers

The expression pattern of RSU1 in different cancers was analyzed by GEPIA based on TCGA and GTEx datasets. It was found that, compared with normal tissues, RSU1 was highly expressed in glioblastoma multiforme (GBM), brain lower grade glioma (LGG), pancreatic adenocarcinoma (PAAD), stomach adenocarcinoma (STAD), and testicular germ cell tumors (TGCT) tissues, whereas a low level of RSU1 was found in kidney chromophobe (KICH) tissues (Figure 1A). However, there was no significant difference in RSU1 expression in colon adenocarcinoma (COAD), although the level of RSU1 in COAD tissues tended to be slightly increased.

Prognostic value of RSU1 in patients with different types of cancer

Further investigation focused on the prognostic role of RSU1 in different types of cancer. Based on the Kaplan-Meier plotter database, survival information was analyzed in patients with GC, breast cancer, ovarian cancer and lung cancer. A high expression level of RSU1 in patients with GC predicted poor OS, with hazard ratios (HR) = 1.36 (1.09-1.7), first progression HR = 1.53 (1.13-2.07) and post-progression survival HR = 1.6 (1.22-2.11) (Figure 2A-C). Regarding breast cancer, the expression level of RSU1 did not affect either the OS or PFS of patients with breast cancer, although significantly poor PPS was found in breast cancer patients with high RSU1 expression (HR = 1.8 (1.26-2.58), Figure 2D-F). In patients with ovarian cancer, the association between RSU1 level and survival was only found in OS [HR = 1.35 (1.09-1.68)], while PFS and PPS were not associated with RSU1 expression (Figure 2G-I). However, a high level of RSU1 predicted good OS in patients with lung cancer, with an HR = 0.56 (0.47-0.66), but no relationship with FP and PPS (Figure 2J-L). The above survival results in different types of cancer suggested that RSU1 may have distinct function in GCs, even in GICs.



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Figure 1 Expression of Ras suppressor 1 in different tumors. Red dots indicate tumors, and green dots are corresponding normal tissue.

RSU1 is prognostic for GIC

To better understand the relevance and underlying mechanisms of RSU1 expression in GC, the relationship between the RSU1 expression and clinical characteristics of GC patients in the Kaplan-Meier plotter database was analyzed through six GC cohorts (GSE14210, GSE15459, GSE22377, GSE29272, GSE51105, GSE62254). Interestingly, in female patients with GC, the expression level of RSU1 was associated with OS, FP and PPS ($P < 0.05$, Table 1). Among different tumor stage parameters, the expression of RSU1 was correlated with large tumor size (T) and predicted poor survival of GC patients with the highest HR = 4.86. However, with or without lymph node metastasis, high RSU1 expression was associated with poor survival in GC, with HRs ranging from 1.35 to 10.98. Regarding distant metastasis, GC patients with increased RSU1 expression predicted shorter OS, and poor FP and PPS. Even HER2 status can affect the prognostic capability of RSU1, giving an HR = 1.46–1.79 in GC patients with negative HER2 status.

To investigate the prognostic value of RSU1 in other GICs, GEO datasets were recruited for further analysis, as no survival information of CRC was found in Kaplan-Meier Plotter. Interestingly, both in GSE17536 and GSE17537, CRC patients with high RSU1 expression tended to have short OS, disease-free survival (DFS), and disease-specific survival (DSS). However, as the sample sizes were limited, statistical significance was only found in GSE17536 in regard to DSS in CRC patients (HR = 1.86, Figure 3).

Pathway enrichment analysis of RSU1-correlated genes

To investigate the potential signaling pathways involved in RSU1-regulated development of GIC, the top 100 RSU1-related genes were collected and analyzed with the KEGG pathways and GO projects, and mapped onto GO for biological process (BP), cellular component (CC), and molecular function (MF) analysis (Figure 4).

The highest enrichment of BP, CC and MF included actin cytoskeleton organization, focal adhesion, extracellular exosome, protein binding and actin binding (Figure 4A-C). In addition, among the top 10 KEGG pathways, endocytosis, Salmonella infection, bacterial invasion of epithelial cells, and tight junction were significantly enriched for RSU1 and RSU1-related genes (Figure 4D). It is reported that the actin cytoskeleton is essential for maintaining cell shape and promoting movement, and plays a key role in tumor invasion and metastasis[17], suggesting that RSU1 may be linked to the tumorigenesis and progression of cancers.

RSU1 expression is associated with immune infiltration levels in GIC

Tumor immune escape is a major obstacle limiting the efficacy of current immunotherapy. It has been reported that actin cytoskeletal remodeling can protect tumor cells from natural killer-mediated cytotoxicity[18]. Thus, RSU1 could affect immune infiltration in GICs based on its association with actin cytoskeletal remodeling.

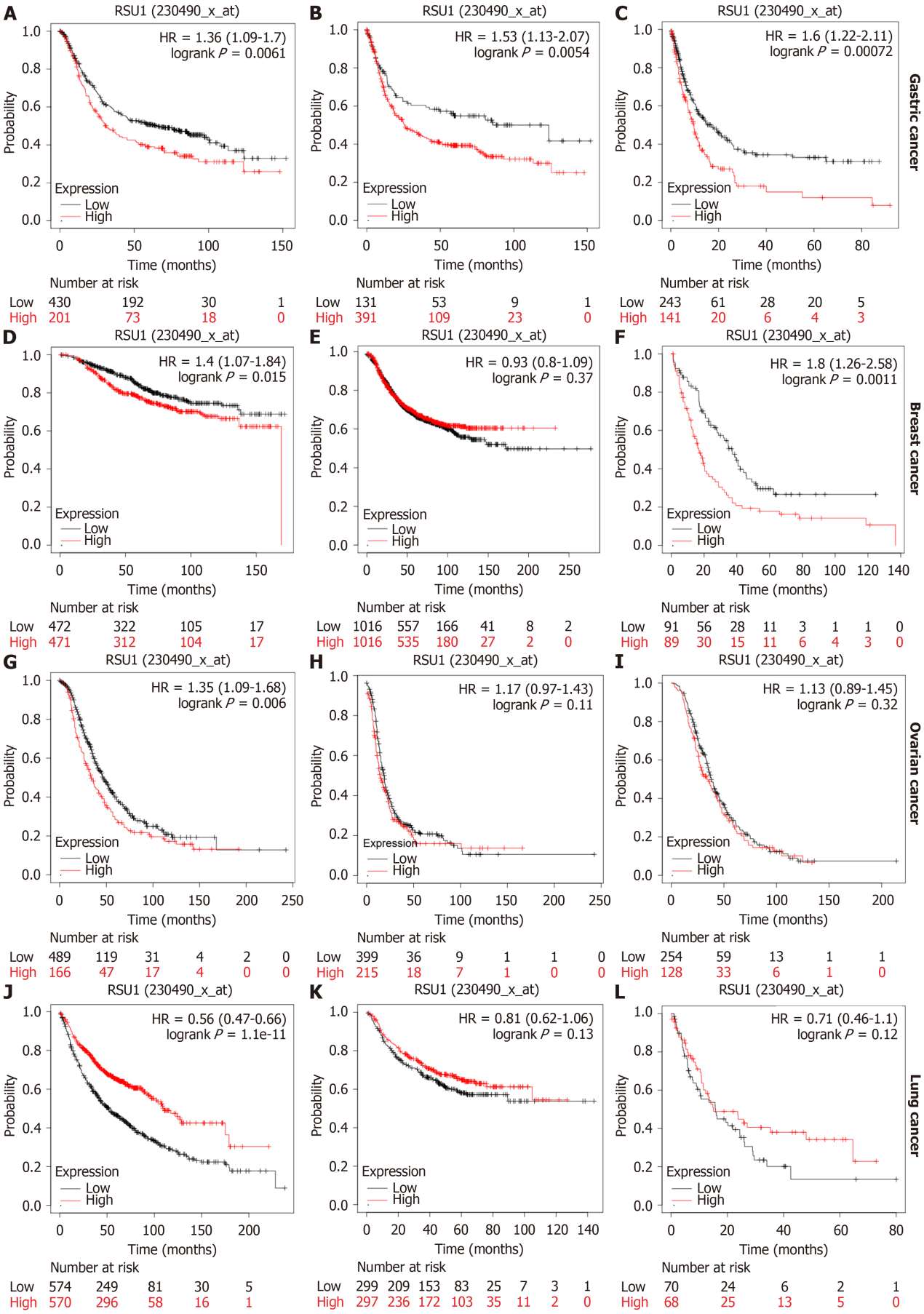
Table 1 Correlation of Ras suppressor 1 expression and prognosis in gastric cancer with various clinicopathological factors

	OS (631)			FP (522)			PPS (384)		
	n	HR	*P* value	*n*	HR	*P* value	*n*	HR	*P* value
Sex									
Female	187	1.88 (1.22-2.89)	[c]	179	1.65 (1.09-2.51)	[a]	127	1.99 (1.21-3.25)	[b]
Male	349	1.36 (0.94-1.97)	0.098	341	1.4 (0.98-2.01)	0.064	256	1.52 (1.09-2.14)	[a]
Stage									
I	62	2.84 (0.63-12.88)	0.16	60	2.7 (0.6-12.26)	0.18	31	2.79 (0.53-14.68)	0.21
II	135	1.47 (0.79-2.75)	0.22	131	0.73 (0.4-1.32)	0.29	105	2.01 (1.04-3.9)	[a]
III	197	1.27 (0.87-1.87)	0.22	186	1.51 (1.02-2.22)	[a]	142	1.2 (0.77-1.86)	0.42
IV	140	1.53 (0.95-2.46)	0.078	141	1.17 (0.74-1.84)	0.51	104	1.79 (1.13-2.83)	[a]
T									
T2	241	1.64 (0.98-2.73)	0.055	239	1.42 (0.88-2.29)	0.15	196	1.57 (0.9-2.72)	0.11
T3	204	1.3 (0.92-1.83)	0.14	204	1.36 (0.95-1.94)	0.091	150	1.36 (0.93-1.99)	0.12
T4	38	3.33 (1.12-9.96)	[a]	39	2.12 (0.85-5.3)	0.1	29	4.68 (1.34-16.32)	[b]
N									
N0	74	2.77 (1.19-6.43)	[a]	72	2.69 (1.16-6.23)	[a]	41	10.98 (2.7-44.62)	[c]
N1-3	422	1.54 (1.18-2.01)	[b]	423	1.35 (1.04-1.74)	[a]	337	1.64 (1.22-2.19)	[c]
M									
M0	444	1.72 (1.2-2.47)	[b]	443	1.63 (1.16-2.31)	[b]	342	1.77 (1.31-2.4)	[c]
M1	56	1.84 (1.02-3.29)	[a]	56	1.44 (0.79-2.6)	0.23	56	2.09 (0.98-4.45)	0.052
HER2 status									
Negative	429	1.46 (1.12-1.91)	[b]	429	1.55 (1.06-2.26)	[a]	283	1.79 (1.28-2.5)	[c]
Positive	202	1.41 (0.95-2.09)	0.086	166	1.46 (0.9-2.38)	0.13	101	1.46 (0.89-2.4)	0.14
Treatment									
Surgery alone	380	1.44 (1.07-1.93)	[a]	375	1.36 (0.97-1.9)	0.071	277	1.82 (1.31-2.52)	[c]
5-FU-based adjuvant	34	1.77 (0.69-4.52)	0.23	34	1.8 (0.77-4.25)	0.17	21	0.39 (0.13-1.18)	0.085
Other adjuvant	76	2.65 (1.09-6.4)	[a]	80	3.46 (1.03-11.67)	[a]	74	2.5 (0.98-6.41)	[a]

[a]$P < 0.05$.
[b]$P < 0.01$.
[c]$P < 0.001$.
5-FU: 5-fluorouracil; FS: First progression; HR: Hazard ratio; OS: Overall survival; PPS: Post-progression survival.

To explore the potential function of RSU1 in GICs, two datasets related to GICs were downloaded and analyzed through ESTIMATE in R software. It is found that along with the increased RSU1 expression level, the StromaScore and ESTIMATEScore were elevated gradually with $P < 0.05$ in CRC and GC (Figure 5A and C). After dividing the patients into high RSU1 *vs* low RSU1 groups, similar results were found in StromaScore and ESTIMATEScore. In the group with high RSU1 levels, StromaScore and ESTIMATEScore were significantly higher compared with the low RSU1 group (Figure 5B and D).

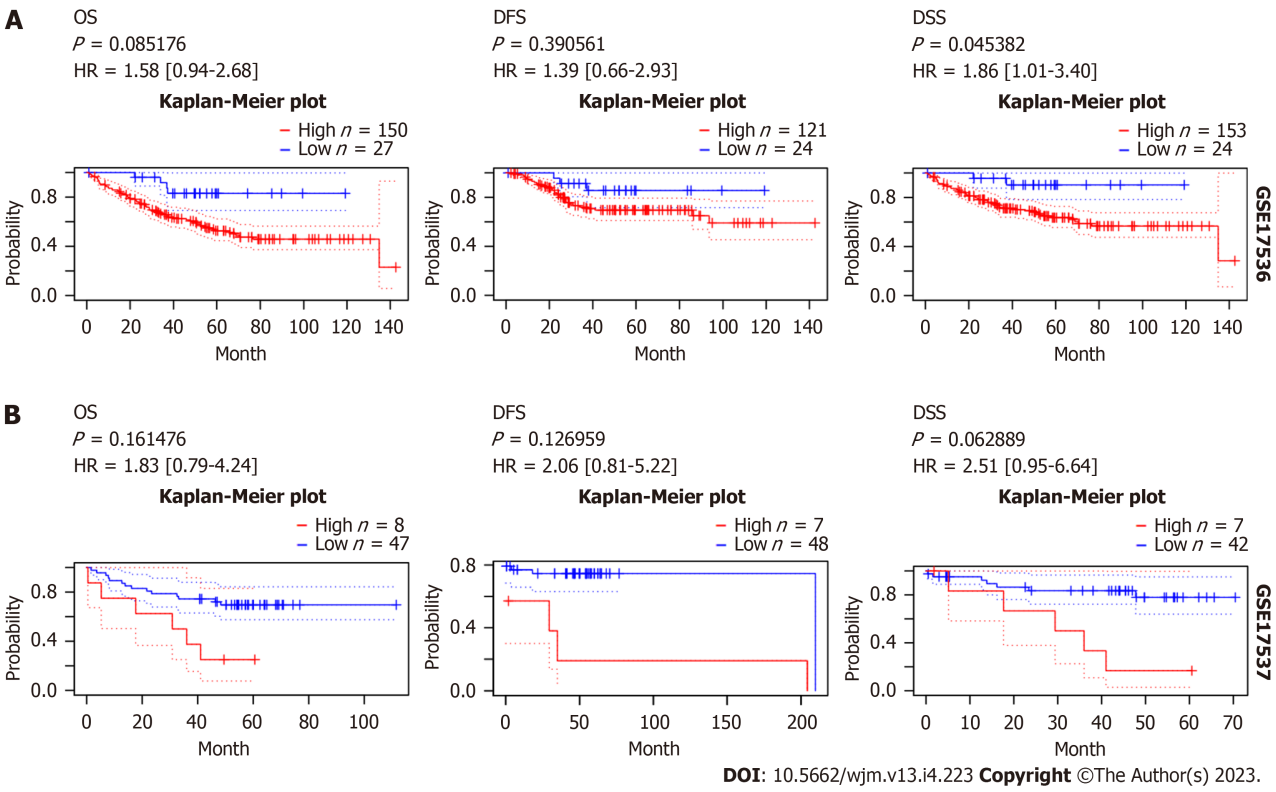
The relationship between RSU1 expression and immune infiltration levels in GIC was explored, revealing that in COAD, RSU1 expression was not significantly correlated with tumor purity (r = -0.089, P = 7.37e-02), CD8+ T cells (r = -0.1, P = 9.74e-02) or B cells (r = -0.03, P = 6.16e-01), but was positively correlated with the infiltration levels of CD4+ T cells (r = 0.36, P = 7.73e-10), macrophages (r = 0.304, P = 2.69e-07), neutrophils (r = 0.12, P = 4.66e-02) and DCs (r = 0.237, P = 7.21e-05) (n = 458) (Figure 6A). Interestingly, in STAD, RSU1 expression was found to be associated with the changed immune infiltration, which was positively correlated with infiltration levels of CD8+ T cells (r = 0.41, P = 8.50e-17), CD4+ T cells (r = 0.235, P = 3.66e-06), macrophages (r = 0.46, P = 2.78e-21), neutrophils (r = 0.309, P = 8.33e-10), and DCs (r = 0.372, P = 6.43e-14) (n = 415), but not for tumor purity (r = -0.062, P = 2.29e-01) and B cells (r = 0.057, P = 2.70e-01) (Figure 6B).



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Figure 2 The prognostic value of Ras suppressor 1 in different tumors. A: Overall survival (OS) in gastric cancers (GC); B: First progression (FP) in

GC; C: Post-progression survival (PPS) in GC; D: OS in breast cancer (BC); E: PFS in BC; F: PPS in BC; G: OS in ovarian cancer (OC); H: PFS in OC; I: PPS in OC; J: OS in lung cancer (LC); K: FP in LC; L: PPS in LC.



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Figure 3 The prognostic value of Ras suppressor 1 in patients with colorectal cancer. A: Overall survival (OS), disease-free survival (DFS) and disease-specific survival (DSS) in GSE17536; B: OS, DFS and DSS in GSE17537.

The clinical trials or vaccine therapies based on immune checkpoint blockade against GBM have not been successful, mainly due to its highly immunosuppressive environment and multiple resistance mechanisms[19]. To compare these findings, GBM was analyzed as control and found that except for macrophage and DC infiltration, expression of RSU1 was not associated with most immune cell infiltration ($P > 0.05$) (Figure 6C). These findings indicate that RSU1 may play a specific role in immune infiltration in the development of GIC, especially through regulating the infiltration of CD4+ T cells, macrophages and DCs.

Correlation between RSU1 level and immune cell biomarkers in GIC

As a relationship between RSU1 Level and immune infiltration was found, biomarkers of different immune cells were characterized to explore the potential function and mechanism of RSU1-related immune infiltration in GIC. The immune cells recruited included CD8+ T cells, T cells, B cells, monocytes, Tumor-associated macrophage (TAMs), M1 and M2 macrophages, neutrophils, natural killer (NK) cells, and DCs, as well as functionally different T cells, such as Th1, Th2, Tfh, Th17, Treg, and exhausted T cells (Table 2). After adjustment for purity, RSU1 expression levels were significantly correlated with 33 out of 57 immune cell biomarkers in COAD, and 48 out of 57 in STAD (Table 2).

Interestingly, the expression levels of biomarkers in most monocyte, TAM, and M2 macrophage marker groups were strongly correlated with RSU1 expression in GIC. It has been reported that the polarization of macrophages from M1 (anti-tumor macrophages) to M2 (pro-tumor macrophages) triggers apoptosis of CD8 T cells, and restricts T cell receptor clustering, all of which contribute to immune escape and promote tumor progression[20]. Specifically, the chemokines of TAMs (CCL-2 and IL10), and M2 (CD163, VSIG4, and MS4A4A) were positively correlated with RSU1 expression in GICs ($P < 0.001$), suggesting a potential role of RSU1 in modulating macrophage polarization in GICs.

High expression of RSU1 was also associated with high infiltration of DCs in GIC, based on its positive association with DC markers, such as HLA-DRA, HLA-DPA1, BDCA-1, BDCA-4 and CD11c ($P < 0.05$). For T cells with different functions in GIC, RSU1 was positively correlated with FOXP3, CCR8, and STAT5B in Treg cells, and associated with CTLA and TIM-3 in T cell exhaustion, indicating the potential role of RSU1 in regulating Treg and T cell exhaustion.

For comparison, GBM was also analyzed for the relationship between RSU1 level and immune cell biomarkers. Only 12 out of 57 immune cell biomarkers were found to be associated with high RSU1 expression in GBM, those being CD115 in monocytes, CD68 in TAMs, COX2 in M1 macrophages, CD11B and CCR7 in neutrophils, BDCA-4 and CD11c in DCs, STAT6 in Th2 T cells, BCL6 in Tfh T cells, STAT3 in Th17 T cells, and STAT5B and TGFβ in Tregs. These findings confirm a potential role of RSU1 in immune escape in GIC through regulating macrophage polarization, DC infiltration and T cell

Table 2 Correlation between Ras suppressor 1 level and immune cell biomarkers in gastrointestinal cancers

Immune cell	Biomarker	COAD				STAD				GBM			
		None		Purity		None		Purity		None		Purity	
		Cor	*P* value	Cor	*P* value	Cor	*P* value	Cor	*P* value	Cor	*P* value	Cor	*P* value
CD8+ T cell	CD8A	0.052	2.64E-01	0.008	8.73E-01	0.306	c	0.305	c	-0.056	4.90E-01	-0.026	7.62E-01
	CD8B	-0.043	3.60E-01	-0.076	1.25E-01	0.158	b	0.151	b	-0.157	5.30E-02	-0.124	1.50E-01
T cell	CD3D	0.034	4.72E-01	-0.009	8.63E-01	0.23	c	0.228	c	-0.140	8.37E-02	-0.114	1.86E-01
	CD3E	0.123	b	0.099	a	0.248	c	0.245	c	-0.042	6.06E-01	0.007	9.38E-01
	CD2	0.176	c	0.168	c	0.304	c	0.307	c	-0.074	3.61E-01	-0.034	6.96E-01
B cell	CD19	0.116	a	0.099	a	0.183	c	0.161	b	0.019	8.14E-01	-0.047	5.86E-01
	CD79A	0.197	c	0.18	c	0.18	c	0.155	b	0.093	2.55E-01	0.133	1.21E-01
Monocyte	CD86	0.255	c	0.258	c	0.361	c	0.375	c	0.024	7.72E-01	0.104	2.28E-01
	CD115 (CSF1R)	0.189	c	0.176	c	0.439	c	0.44	c	0.200	a	0.302	c
TAM	CCL2	0.352	c	0.337	c	0.348	c	0.355	c	0.034	6.74E-01	0.083	3.35E-01
	CD68	0.096	a	0.094	5.76E-02	0.17	c	0.169	c	0.169	a	0.275	b
	IL10	0.21	c	0.204	c	0.384	c	0.406	c	-0.043	5.98E-01	0.036	6.78E-01
M1 Macrophage	INOS (NOS2)	-0.196	c	-0.196	c	0.035	4.75E-01	0.061	2.36E-01	0.049	5.50E-01	0.082	3.39E-01
	IRF5	0.216	c	0.219	c	0.108	a	0.093	7.03E-02	0.040	6.26E-01	0.094	2.74E-01
	COX2 (PTGS2)	0.084	7.40E-02	0.039	4.31E-01	0.186	c	0.183	c	0.198	a	0.254	b
M2 Macrophage	CD163	0.187	c	0.168	c	0.395	c	0.397	c	0.155	5.51E-02	0.213	a
	VSIG4	0.18	c	0.158	b	0.401	c	0.421	c	0.018	8.25E-01	0.101	2.39E-01
	MS4A4A	0.221	c	0.204	c	0.421	c	0.431	c	0.060	4.58E-01	0.166	5.21E-02
Neutrophils	CD66B (CEACAM8)	-0.024	6.15E-01	-0.013	7.98E-01	0.029	5.57E-01	0.034	5.08E-01	0.033	6.87E-01	0.044	6.11E-01
	CD11B (ITGAM)	0.136	b	0.13	b	0.392	c	0.392	c	0.233	b	0.319	c
	CCR7	0.17	c	0.16	b	0.322	c	0.325	c	0.226	b	0.254	b
Natural killer cell	KIR2DL1	-0.024	6.08E-01	-0.057	2.51E-01	0.143	b	0.145	b	-0.025	7.62E-01	-0.027	7.52E-01
	KIR2DL3	-0.076	1.06E-01	-0.091	6.78E-02	0.094	5.45E-02	0.083	1.07E-01	-0.022	7.88E-01	-0.052	5.48E-01
	KIR2DL4	-0.072	1.23E-01	-0.11	a	0.005	9.25E-01	0.003	9.61E-01	-0.009	9.11E-01	0.009	9.13E-01
	KIR3DL1	-0.049	2.92E-01	-0.073	1.40E-01	0.142	b	0.127	a	0.042	6.06E-01	0.018	8.34E-01
	KIR3DL2	-0.01	8.31E-01	-0.063	2.04E-01	0.146	b	0.147	b	-0.086	2.93E-01	-0.060	4.86E-01
	KIR3DL3	-0.086	6.63E-02	-0.093	6.04E-02	-0.078	1.12E-01	-0.064	2.16E-01	-0.118	1.48E-01	-0.107	2.12E-01
	KIR2DS4	-0.015	7.48E-01	-0.026	5.99E-01	0.051	3.03E-01	0.044	3.95E-01	-0.001	9.88E-01	0.003	9.72E-01
Dendritic cell	HLA-DPB1	0.051	2.73E-01	0.024	6.27E-01	0.287	c	0.291	c	0.032	6.91E-01	0.108	2.08E-01
	HLA-DQB1	0.056	2.29E-01	0.037	4.55E-01	0.152	b	0.153	b	0.152	6.11E-02	0.234	b
	HLA-DRA	0.125	b	0.103	a	0.268	c	0.27	c	-0.049	5.51E-01	0.014	8.73E-01
	HLA-DPA1	0.16	c	0.142	b	0.255	c	0.256	c	0.032	6.91E-01	0.108	2.08E-01
	BDCA-1 (CD1C)	0.29	c	0.291	c	0.303	c	0.302	c	-0.030	7.10E-01	0.025	7.70E-01
	BDCA-4 (NRP1)	0.348	c	0.37	c	0.523	c	0.52	c	0.376	c	0.462	c
	CD11c (ITGAX)	0.156	c	0.15	b	0.341	c	0.348	c	0.081	3.19E-01	0.099	2.49E-01
Th1	T-bet (TBX21)	0.069	1.39E-01	0.06	2.27E-01	0.266	c	0.264	c	0.237	b	0.258	b
	STAT4	0.159	c	0.148	b	0.301	c	0.294	c	-0.091	2.63E-01	-0.043	6.18E-01
	STAT1	0.197	c	0.182	c	0.189	c	0.177	c	0.078	3.38E-01	0.075	3.84E-01
	IFN-γ (IFNG)	0.023	6.28E-01	0.009	8.53E-01	0.098	a	0.091	7.73E-02	0.120	1.40E-01	0.130	1.30E-01

	TNF-α (TNF)	0.118	[a]	0.098	[a]	0.104	[a]	0.071	1.67E-01	0.022	7.83E-01	0.026	7.67E-01
Th2	GATA3	0.176	[c]	0.183	[c]	0.305	[c]	0.304	[c]	0.125	1.24E-01	0.143	9.67E-02
	STAT6	0.006	8.92E-01	0.01	8.44E-01	0.224	[c]	0.219	[c]	0.289	[c]	0.353	[c]
	STAT5A	0.155	[c]	0.172	[c]	0.431	[c]	0.426	[c]	0.071	3.81E-01	0.104	2.24E-01
	IL13	0.124	[b]	0.099	[a]	0.151	[b]	0.153	[b]	0.021	7.93E-01	0.029	7.39E-01
Tfh	BCL6	0.145	[b]	0.143	[b]	0.388	[c]	0.367	[c]	0.238	[b]	0.232	[b]
	IL21	0.074	1.13E-01	0.06	2.31E-01	0.133	[b]	0.12	[a]	0.023	7.73E-01	-0.026	7.67E-01
Th17	STAT3	0.318	[c]	0.327	[c]	0.426	[c]	0.411	[c]	0.404	[c]	0.394	[c]
	IL17A	0.055	2.42E-01	0.057	2.51E-01	-0.124	[a]	-0.133	[b]	-0.077	3.42E-01	-0.082	3.42E-01
Treg	FOXP3	0.218	[c]	0.214	[c]	0.224	[c]	0.223	[c]	0.065	4.24E-01	0.073	3.98E-01
	CCR8	0.317	[c]	0.327	[c]	0.341	[c]	0.336	[c]	0.052	5.26E-01	0.082	3.38E-01
	STAT5B	0.401	[c]	0.413	[c]	0.499	[c]	0.491	[c]	0.218	[b]	0.179	[a]
	TGFβ (TGFB1)	0.114	[a]	0.106	3.28E-02	0.418	[c]	0.406	[c]	0.325	[c]	0.387	[c]
T cell (exhausted)	PD1 (PDCD1)	-0.016	7.28E-01	-0.05	3.16E-01	0.205	[c]	0.202	[c]	0.085	2.96E-01	0.132	1.23E-01
	CTLA4	0.1	[a]	0.092	6.43E-02	0.17	[c]	0.164	[b]	0.030	7.12E-01	0.060	4.89E-01
	LAG3	-0.001	9.91E-01	-0.041	4.04E-01	0.202	[c]	0.196	[c]	0.116	1.52E-01	0.126	1.42E-01
	TIM-3 (HAVCR2)	0.223	[c]	0.22	[c]	0.38	[c]	0.391	[c]	0.046	5.76E-01	0.106	2.16E-01
	GZMB	0.028	5.53E-01	0.007	8.89E-01	0.134	[b]	0.132	[b]	-0.009	9.15E-01	0.043	6.20E-01

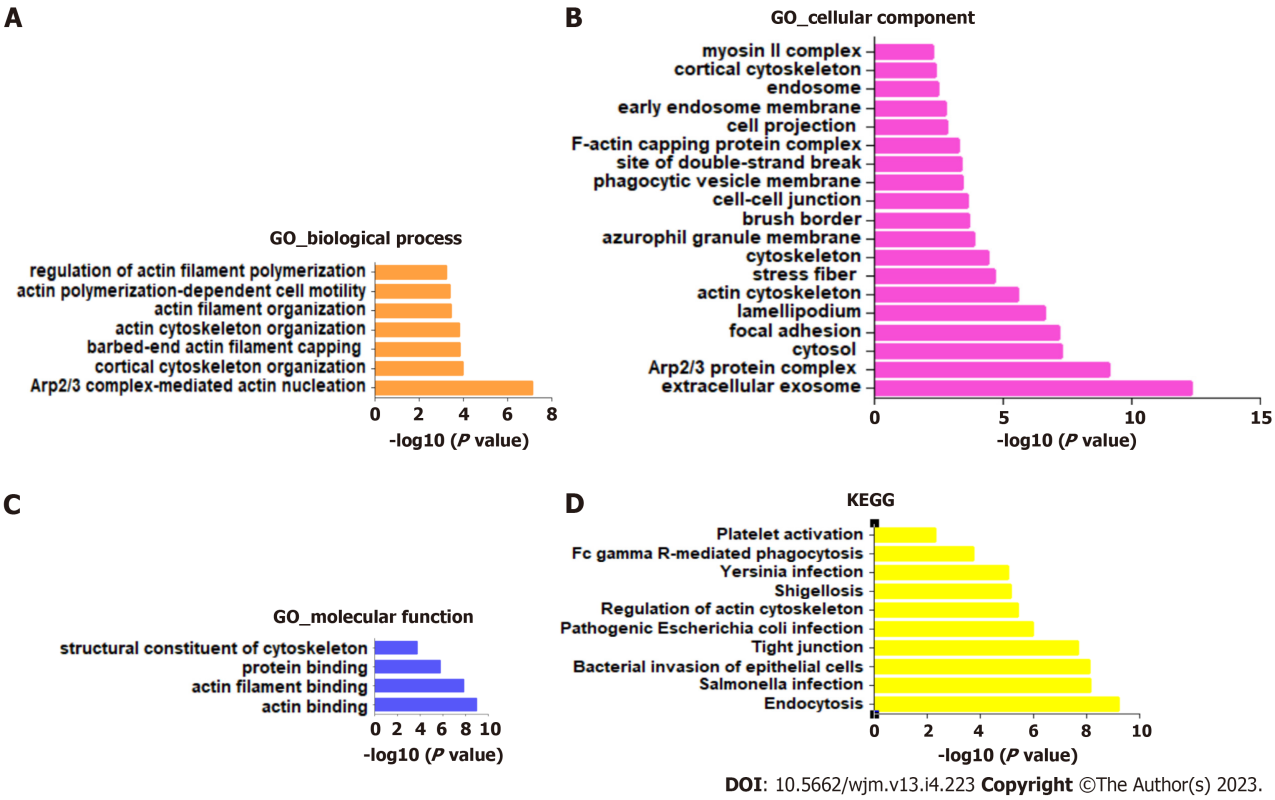
[a]$P < 0.05$.
[b]$P < 0.01$.
[c]$P < 0.001$.
COAD: Colon adenocarcinoma; STAD: Stomach adenocarcinoma; GBM: Glioblastoma multiforme.

exhaustion, resulting in promotion of tumor progression.

DISCUSSION

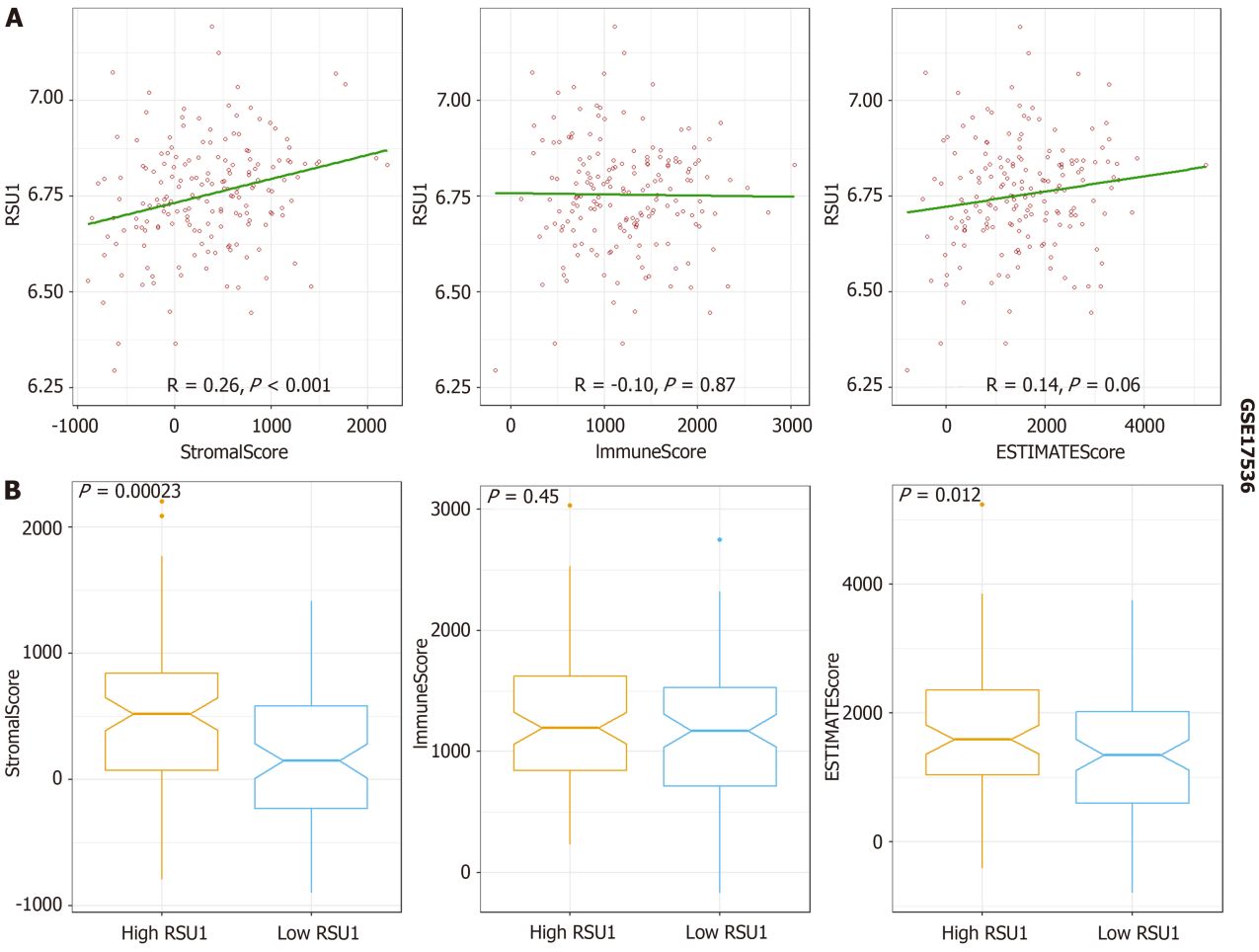
The actin cytoskeleton mediates many essential biological functions in all eukaryotic cells. In addition to providing the structural framework necessary to determine cell shape and polarity, the actin cytoskeleton, especially its dynamics, is also closely related to cell movement, division, adhesion, and phagocytosis[21]. The actin cytoskeleton also mediates many pathological functions, such as playing a key role in tumor cell invasion and metastasis[22,23]. Among diverse different regulating factors, RSU1 participates in actin cytoskeletal remodeling, which is essential for the development of cancers. Current research reported the potential role of RSU1 in regulating immune escape in GICs through mediating the actin cytoskeleton.

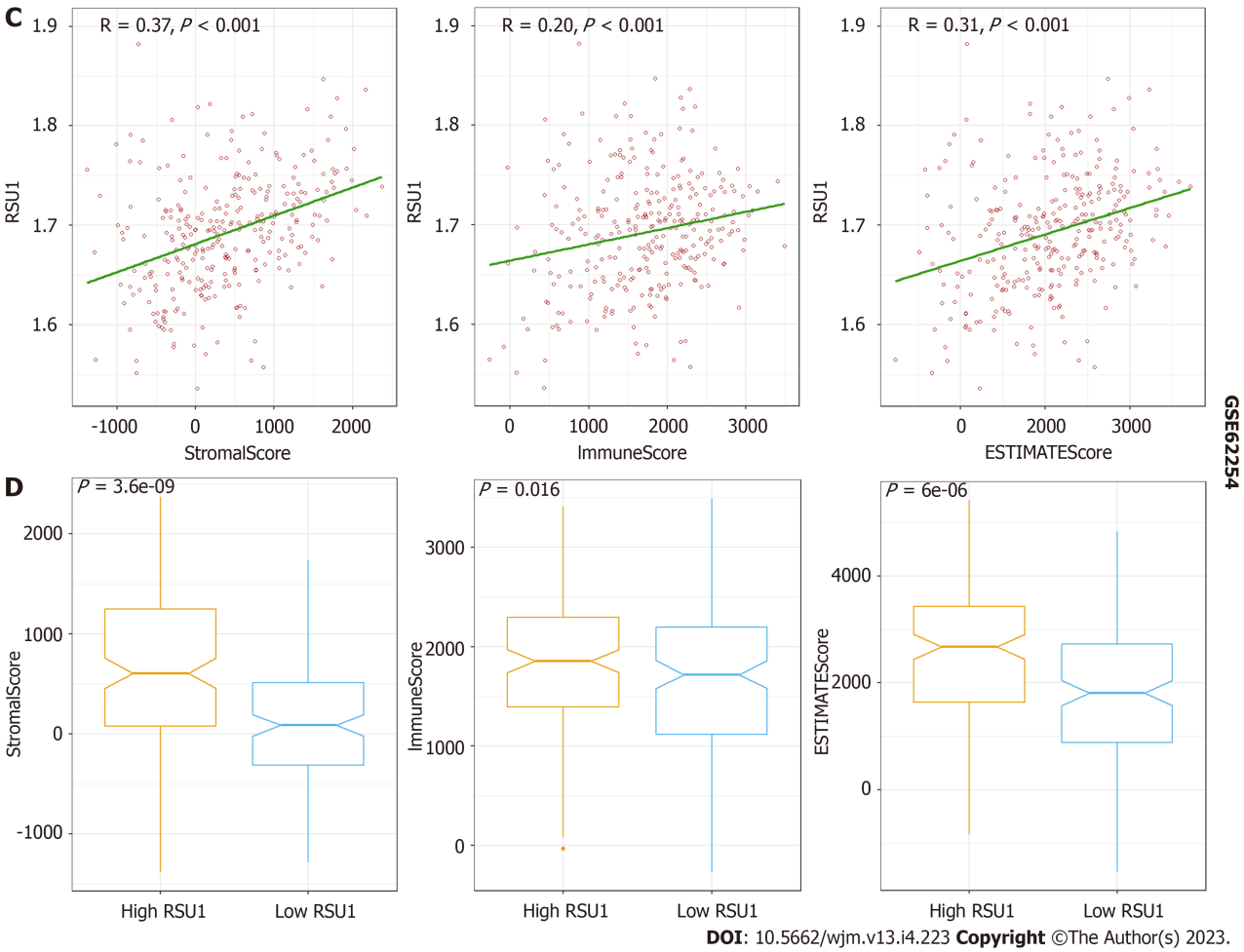
It is found that RSU1 is involved in tumor progression in various cancer cell lines *in vitro*, but *in vivo* studies of the role of RSU1 in cancer is still missing. In this study, the expression pattern of RSU1 in different cancers was examined. Although at the mRNA level, RUS1 Level is associated only with GBM, LGG, PAAD, STAD, and TGCT, in patients with GICs, the high level of RSU1 is predicted to promote the development of gastric and colorectal cancers. In breast cancer studies, Vasaturo *et al*[24] demonstrated that overexpression of RSU1 in Michigan Cancer Foundation-7 (MCF-7) breast cancer cells induced p21 activation and reduced cancer cell proliferation by inhibiting cyclin-dependent kinase, suggesting that RSU1 acts as a tumor suppressor gene in breast cancer, but another study revealed that RSU1 is upregulated at both mRNA and protein levels in more aggressive and highly aggressive MDA-MB-231 breast cancer cells compared to non-invasive MCF-7 breast cancer cells[25]. Consistent with the above study, Christou *et al*[26] reported that high RSU1 expression is associated with poor breast cancer survival. In hepatocellular carcinoma, RSU1 expression has also been found to be elevated in more aggressive HepG2 hepatocellular carcinoma cells compared to non-metastatic Alexander cells[13], and RSU1 promotes invasion of hepatocellular carcinoma, similar to breast cancer cells. In glioblastoma, the more aggressive cells (A172 and U87-MG) also exhibit increased expression of RSU1 compared to less aggressive glioma cell lines (H4 and SW1088)[11]. In aggressive liver, breast cancer and brain cells, RSU1 is upregulated, and the blocking of its expression efficiently inhibits cell migration and invasion. Based on the previous reports and current study, the oncogenic role of RSU1 has emerged in different types of malignancies, especially in GICs. Here, we report a study supporting the role of RSU1 in GC. RSU1 mRNA expression is significantly higher in STAD tissues compared with adjacent normal tissues, and RSU1 expression is associated with poor prognosis of GC patients in the Kaplan-Meier plotter databases. Also, high RSU1 expression is associated with poorer OS, poorer FP, and poorer PPS in GC. However,



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Figure 4 Pathway enrichment analysis of Ras suppressor 1-related genes. A: Biological process; B: Cellular component; C: Molecular function; D: Kyoto Encyclopedia of Genes and Genomes.





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Figure 5 ESTIMATE analyses of Ras suppressor 1 with StromaScore, ImmuneScore and ESTIMATEScore. A and B: Correlation with Ras suppressor 1 (RSU1) level in colorectal cancer; C and D: Correlation with RSU1 level in GC.

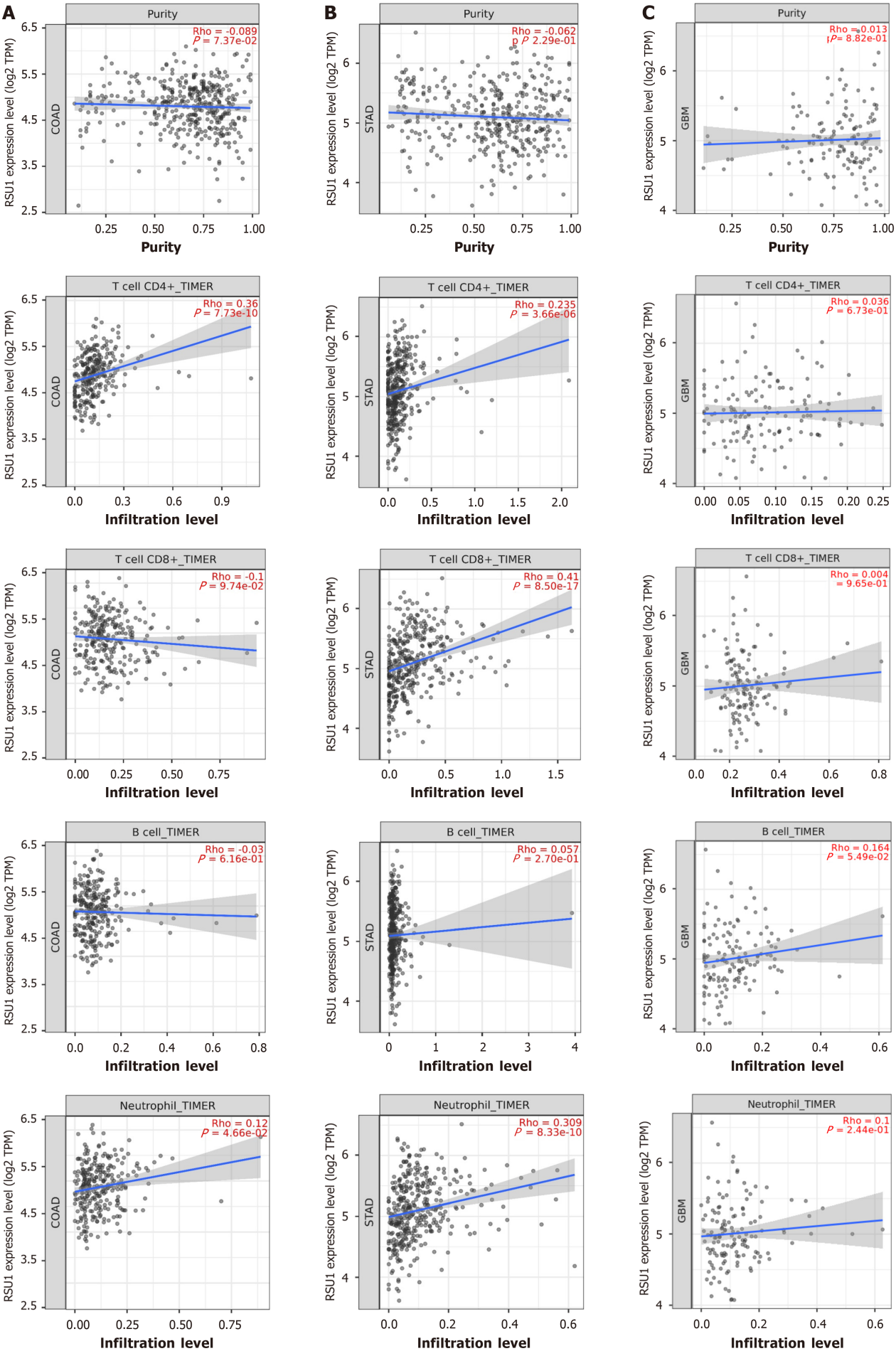
the underlying mechanism is unclear.

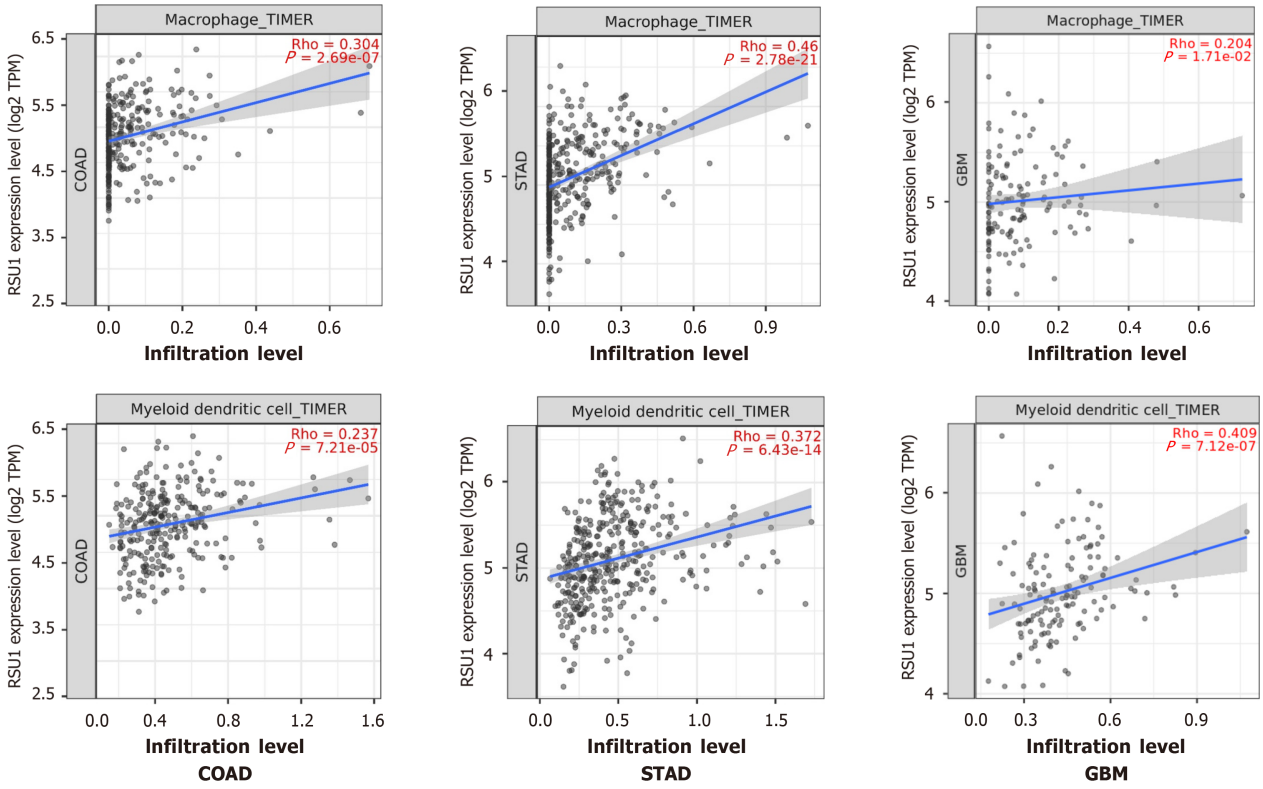
Recent studies have shown that actin cytoskeletal remodeling is also closely related to the function of various immune cells (including T cells, B cells, and macrophages), which are involved in the formation of immune synapses and the development and maturation of T cells[27,28]. Actin cytoskeletal remodeling is also involved in the function of B cells and chemotaxis and phagocytosis in macrophages, through B cell antigen presentation by regulating BCR signaling[29,30]. Interestingly, actin is increased in resistant tumor cells, and the actin response is related to the protection of cancer cells against NK cell attack[18]. However, the regulatory function of tumor cell RSU1 on immune cells has not been studied yet. To elaborate the relationship between RSU1 and different immune cells, we found RSU1 expression is positively correlated with infiltration levels of CD4+ T cells, macrophages, neutrophils, and DCs in STAD. RSU1 plays a crucial role in immune escape of STAD microenvironments through macrophage polarization, DC infiltration, and T cell exhaustion, resulting in promotion of tumor progression.

The results of two clinical studies, CheckMate-649 and KEYNOTE-062, predicted that the response to immunotherapy cannot be judged solely by traditional indications, such as PD-L1 or tumor mutational burden. Since then, a large number of tumor patients with PD-L1 negative or low TMB have produced unusually long-lasting responses. Therefore, the development of new markers to predict immunotherapeutic response needs further investigation. PD-1, PD-L1 and CTLA4 are the main targets of immunotherapy. The present study shows that RSU1 is positively correlated with the expression of PD1 and CTLA4 in GC, and therefore RUS1 may be a potential prognostic factor for predicting the efficacy of immunotherapy.

CONCLUSION

In conclusion, our study provides insights into the potential role of RSU1 in tumor immunology and its prognostic value. RSU1 is associated with prognosis and TIICs in patients with different types of cancer, especially COAD and STAD, including CD4+ T cells, macrophages, neutrophils, and DCs. Higher RSU1 expression is associated with poor prognosis of GC patients and RSU1 plays a crucial role in immune escape of STAD microenvironments through macrophage polarization, DCs infiltration, and T cell exhaustion, and promotes tumor progression. RSU1 mRNA level is correlated


A
B
C
Purity
Rho = -0.089
P = 7.37e-02
Rho = -0.062
P = 2.29e-01
Rho = 0.013
P = 8.82e-01
COAD
STAD
GBM
RSU1 expression level (log2 TPM)
Purity
T cell CD4+_TIMER
Rho = 0.36
P = 7.73e-10
Rho = 0.235
P = 3.66e-06
Rho = 0.036
P = 6.73e-01
Infiltration level
T cell CD8+_TIMER
Rho = -0.1
P = 9.74e-02
Rho = 0.41
P = 8.50e-17
Rho = 0.004
P = 9.65e-01
B cell_TIMER
Rho = -0.03
P = 6.16e-01
Rho = 0.057
P = 2.70e-01
Rho = 0.164
P = 5.49e-02
Neutrophil_TIMER
Rho = 0.12
P = 4.66e-02
Rho = 0.309
P = 8.33e-10
Rho = 0.1
P = 2.44e-01



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Figure 6 Correlation between Ras suppressor 1 expression and immune infiltration levels in COAD, STAD and GBM using TIMER2.0. A: COAD; B: STAD; C: GBM.

with prognosis and immune infiltration level in GIC, suggesting that RSU1 can be used as a biomarker of prognosis.

ARTICLE HIGHLIGHTS

Research background

At present, gastrointestinal cancers (GICs) in clinical immunotherapy effect is not significant, this study found that Ras suppressor 1 (RSU1) participation in the immune escape process may affect the efficacy of immunotherapy, providing a new target for improving the efficacy of immunotherapy.

Research motivation

The main topic of this study is to explore the role of RSU1 in GICs. Currently, GICs have no significant effect on immunotherapy, and this study found that RSU1 can affect the efficacy of immunotherapy through immune escape, providing a way to improve the efficacy of immunotherapy.

Research objectives

RSU1 is a promising prognostic biomarker reflecting the level of GICs immunoinfiltration and a potential therapeutic target by improving the immune response. At present, this study needs clinical data for verification.

Research methods

We evaluated differential expression of RSU1 in different tumors and their corresponding normal tissues by exploring Gene Expression Profiling Interactive Analysis datasets. The prognostic relationship between RSU1 expression and GICs patients was evaluated using Kaplan-Meier plotter and PrognoScan. Then, RSU1 related genes were screened and functional enrichment was performed by DAVID. Tumor Immune Estimation Resource (TIMER) was used to further characterize the correlation between RSU1 and tumor-infiltrating immune cells. In addition, the correlation between RSU1 and immune cell surface molecules was analyzed by TIMER.

Research results

Our study reveals the potential role of RSU1 in tumor immunology and its prognostic value. RSU1 is involved in immune escape of tumor microenvironment through macrophage polarization, dendritic cells infiltration, T cell depletion and other pathways to promote tumor progression. RSU1 can be used as a prognostic biomarker to provide therapeutic target

for improving the efficacy of immunotherapy.

Research conclusions

The new theories that this study proposes is RSU1 plays a crucial role in immune escape and thus the efficacy of immunotherapy in gastrointestinal cancers. The new methods that this study proposed is the immune status was expressed by analyzing the expression of immune-related factors in the online database.

Research perspectives

This is the first comprehensive analysis of the potential role and prognostic value of the RSU1 in diverse gastrointestinal cancers. However, the current research is limited, and future studies need to further explore the molecular mechanism of RSU1 in regulating the oncogenesis and development of GICs.

FOOTNOTES

Author contributions: Xu Y and Liu J contributed to the study conception and design. Material preparation, data collection and analysis were performed by Xu Y, Hou YY, Wu Z, Fang ZX, and Wu HT; The first draft of the manuscript was written by Xu Y and all authors commented on previous versions of the manuscript; As the corresponding author, Liu J is the general person in charge of the project, responsible for the project funding, design, writing and checking of the article; All authors read and approved the final manuscript.

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Institutional review board statement: In current manuscript, NO-animal experiment was conducted, or NO-Human Participants was involved. So, we did not apply the approval form or document from the committee. Meanwhile, the ARRIVE guidelines were not appropriate for this article.

Conflict-of-interest statement: No conflict of interest is claimed by any author.

Data sharing statement: No additional data are available.

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Country/Territory of origin: China

ORCID number: Ya Xu 0009-0009-8438-9829; Yan-Yu Hou 0000-0002-4249-8770; Zheng Wu 0000-0002-1393-7586; Ze-Xuan Fang 0000-0002-6100-9012; Hua-Tao Wu 0000-0002-1640-6094; Jing Liu 0000-0002-7483-4572.

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L-Editor: A
P-Editor: Xu ZH

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Retrospective Cohort Study

Role of the phase angle in the prognosis of the cirrhotic patient: 15 years of follow-up

Letícia Pereira Pinto, Claudio Augusto Marroni, Juliana Czermainski, Maria Luiza Fernandes Dahlem, Randhall B Carteri, Sabrina Alves Fernandes

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Letícia Pereira Pinto, Claudio Augusto Marroni, Juliana Czermainski, Sabrina Alves Fernandes, Department of Hepatology, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre 90050-170, Brazil

Maria Luiza Fernandes Dahlem, Department of Veterinary Medicine, Universidade Federal do Rio Grande do Sul, Porto Alegre 91540-000, Brazil

Randhall B Carteri, Department of Nutrition, Centro Universitário Metodista IPA, Porto Alegre 90420-060, Brazil

Randhall B Carteri, Department of Nutrition, Centro Universitário Cesuca, Cachoerinha 94935-630, Brazil

Corresponding author: Sabrina Alves Fernandes, PhD, Research Scientist, Teacher, Hepatology, Universidade Federal de Ciências da Saúde de Porto Alegre, Sarmento Leite 245, Porto Alegre 90050-170, Brazil. sabrinaafernandes@gmail.com

Abstract

BACKGROUND

In 2019, cirrhosis accounted for 2.4% of global deaths. The projection for 2030 is an increase in this index. In recent years, hospitalization costs have escalated by 36% for compensated cirrhosis and 24% for decompensated cirrhosis. Therefore, it is necessary to identify a tool capable of predicting the mortality of these patients according to their clinical condition and consequently extending their survival time. Different studies have shown that the phase angle (PA) can be a feasible method in clinical practice, with the potential to guide assertive patient management in the therapeutic of chronic liver disease.

AIM

To evaluate the prognostic role of PA in cirrhotic patients over a 15-year follow-up period.

METHODS

Retrospective cohort study with 129 cirrhotic patients of both sexes over 18 years old. Diagnosis of cirrhosis by liver biopsy. The first year of data collection was 2007, and data regarding outcomes was collected in 2023. Data were gathered from medical records, such as esophageal varices (EV), EV bleeding, ascites,

spontaneous bacterial peritonitis (SBP), encephalopathy, laboratory findings and PA. The cut-off value for the PA was 5.4°, a value described in 2012 by Fernandes *et al* for 129 patients evaluated in this study and the cut-off points for the Brazilian population presented in percentiles (P), as described by Mattiello *et al*. The mortality was assessed using the PA percentile through Kaplan-Meier curves and multivariate binary logistic regression models.

RESULTS

Patients were divided into two groups according to the PA 5.4th (PA > 5.4°, n = 40; PA ≤ 5.4°, n = 89) PA percentile (< P50, n = 56; ≥ P50 n = 73). The percentile classification was more accurate in identifying long-term deaths than the 5.4° PA. Patients with < P50 had a higher number of relevant complications such as ascites, SBP, liver encephalopathy and HCC. PA is strongly correlated with serum albumin (P < 0.001), International Normalized Ratio (P = 0.01), total bilirubin (P = 0.02) and direct bilirubin (P = 0.003). PA is correlated with survival time (P < 0.001) and length of stay (P = 0.02). Logistic regression analysis shows that an increase of 1° in PA enlarges the cirrhotic patient's chance of survival by 17.7%.

CONCLUSION

PA is a good predictor of morbidity and mortality for cirrhotic patients. The PA by percentile showed greater sensitivity in predicting mortality compared to the cut-off point of 5.4°.

Key Words: Liver cirrhosis; Phase angle; Prognosis; Liver transplantation; Electrical bioimpedance

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Core Tip: Cirrhosis is characterized by the destruction of hepatic cells, resulting in metabolic alterations that compromise the body's homeostasis. This has a detrimental effect on the patient's clinical condition, negatively impacting their prognosis. Identifying a parameter capable of predicting relevant events is essential for a more assertive approach, reducing mortality and promoting higher life quality. This study aimed to assess the prognostic role of Phase Angle (PA) in cirrhotic patients. One hundred and twenty-nine patients were included, concluding that PA is a promising tool in predicting the clinical condition of chronic liver disease.

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INTRODUCTION

In 2019, cirrhosis was associated with 2.4% of global deaths. The main etiology of cirrhosis during that period was hepatitis C virus infection, followed by alcohol-related liver disease. However, with advancements in the management of viral hepatitis and the increase in obesity levels and alcohol consumption, the epidemiology of cirrhosis is changing, leading to a growing number of cases associated with Metabolic Associated Steatotic Liver Disease (MASLD) and alcohol, an epidemiological situation that results in a reduced mortality rate for other etiologies[1]. It is estimated that mortality from advanced chronic liver disease will more than double between 2016 and 2030[2].

Cirrhosis has a peculiar pathophysiological characteristic, an increase in the body's energy demand, which results in increased depletion of muscle and hepatic glycogen, increased degradation and protein needs, increased lipoperoxidation due to oxidative stress, and activation of the pro-inflammatory cascade, favoring disease progression and development of complications[3].

This disruption of body homeostasis in cirrhosis, regardless of etiology, leads to cellular damage in terms of integrity and functionality, resulting from parenchymal rearrangement, which causes vascular alterations, portal hypertension, and hepatocellular dysfunction. This pathophysiological characteristic substantially impacts the prognosis of cirrhotic patients[4].

Systematically monitoring the progression of chronic liver disease is a complex task for many healthcare services, as it has numerous limitations, such as being an invasive and costly method and discrepancies in the collected data among professionals. Therefore, there is an urgent need to explore new tools, such as a temporal prognostic index of chronic liver disease based on the natural history of the disease.

Currently, it is possible to assess cellular structure through phase angle (PA). PA is derived from the relationship between resistance and reactance, reflecting the conductivity of electric current through body cells. The value of PA expressed in degrees, is measured by the bioelectrical impedance analysis device[5].

Several studies refer to PA as a prognostic indicator because it can measure damage to cellular integrity and functionality resulting from diseases, compromised nutritional status, and/or hydration status. Thus, it is widely used as a guiding parameter in managing various diseases[6-10].

Therefore, we conducted a cohort study of cirrhotic patients with different etiologies to capture longitudinal trajectories over 15 years. We analyzed the association between PA and the two main outcomes, death and liver transplantation. Additionally, we evaluated the correlation of PA with laboratory parameters of liver function, survival curves of the study population according to PA values, and associations of PA with the main complications of cirrhosis, such as ascites, encephalopathy, spontaneous bacterial peritonitis (SBP), and bleeding esophageal varices.

This study suggests that the segmented and systematic use of PA in routine evaluation of cirrhotic patients can provide important information about the patient's health condition at the time of assessment, enabling early and effective therapeutic intervention to extend the survival time of cirrhotic patients while improving their quality of life.

MATERIALS AND METHODS

Study population

A retrospective cohort study was conducted, enrolling cirrhotic patients of both sexes, over 18 years old, treated at the gastroenterology clinic of a hospital complex in Porto Alegre, Brazil. The diagnosis of cirrhosis was established through histological analysis of liver biopsy, imaging tests, and biological markers. Patients with intestinal malabsorption, acquired immunodeficiency syndrome, chronic kidney failure, those on enteral diet, upper limb neuromuscular alterations, chronic pancreatitis, chronic diarrhea, and mental and/or cognitive disorders were excluded from the study. For survival analysis, patients who underwent transplantation during the follow-up were included until the moment of transplantation. This study was conducted in accordance with the Helsinki Declaration and was approved by the ethics and research committee of the Federal University of Health Sciences of Porto Alegre, RS, Brazil, under the number 5203619. All participants signed the Informed Consent Form in advance.

Data collection

Data collection in the first year occurred in January 2007, and data regarding the outcomes were gathered in January 2023. Demographic data such as age and sex were collected. The clinical data on patient outcomes included the number of hospitalizations, ascites, paracentesis, encephalopathy, VE, SBP, and hepatocellular carcinoma (HCC). The staging of cirrhosis was assessed through the Child-Pugh and Model for End-Stage Liver Disease (MELD) scores.

The laboratory tests taken into consideration were those performed up to three months before or after the evaluation, which included: Albumin, aspartate aminotransferase (AST or TGO), alanine aminotransferase (ALT or TGP), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP or FA), bilirubin levels, and International Normalized Ratio (INR). The duration of hospitalization was calculated based on the admission date and discharge date.

Phase angle analysis

The PA data was collected using tetrapolar electrical bioimpedance from the Biodynamics 450 brand. For the test, participants were instructed to remove all metal objects that could interfere with the examination. The patients were positioned in a supine position with the palms of their hands facing down and their arms along their bodies; electrodes were placed on the middle finger and wrist of the right hand and the ankle and middle finger of the right foot. The PA was established using the following formula: PA = tangent arc (Xc/R) × 180/π.

The PA was categorized according to two references for analysis of the outcomes of death and transplantation: Fernandes[7], which establishes the cut-off point for PA at 5.4°. Thus, a PA score of 5.4 points indicates a suboptimal outcome for all statistical evaluations of the study. The additional reference used was Mattiello[11], through their epidemiological study, which establishes cut-off points of PA for the Brazilian population by percentiles (p) p5, p25, p50, p75, p95. The PA value by percentile is distributed considering the age and sex of the studied sample. A PA in the range of the p50 represents the average normality; hence, a p below 50 (< P50) represents a poor prognosis.

Statistical analysis

A general description of the data gathered is provided through simple and relative frequencies. Data normality was assessed using the Shapiro-Wilk test, and the data were compared using the student's t-test for independent samples. Pearson's correlation was used to evaluate potential interactions between different variables. Survival analysis was performed using the Kalan-Meier method, using PA values. Binary logistic regression was also performed to assess the predictive potential of specific variables with the patient's outcome. Significant values were considered when $P < 0.05$. All data were analyzed using the statistical program Statistical Package for Social Sciences version 22.0.

RESULTS

The study assessed 129 cirrhotic patients with an average age of 56.32 (SD 56.32 ± 11.25), with a predominance of male sex (56.6%). The prevailing etiology was hepatitis C virus (43.40%). Other patient characteristics included in the study are described in Table 1.

The clinical condition of the patients was determined by the Child-Pugh score, where 65.89% were classified as A, 25.59% as B, and 8.52% as C. The MELD score showed an average score of 10.70 (SD 10.70 ± 4.70).

Table 1 Characterization of the sample, *n* (%)

Variáveis	Total, *n* = 129
Age (yr), mean ±SD	56.32 ± 11.25
Gender	
Male	73 (56.6)
Female	56 (43.4)
Weight (kg), mean ± SD	74.46 ± 14.69
Height (m), mean ± SD	164.43 ± 8.97
Phase angle, mean ± SD	6.62 ± 2.95
Etiology Cirrhosis	
Alcohol	31 (24)
Autoimmune	5 (3.90)
Nash	5 (3.90)
Others	16 (12.5)
Virus C	56 (43.40)
Virus C+ alcohol	12 (9.30)
Virus B	4 (3.10)
Meld, mean ± SD	10.98 ± 4.69
Classification Child-Turcotte-Pugh	
Child A	85 (65.89)
Child B	33 (25.59)
Child C	11 (8.52)

NASH: Non-alcoholic steatohepatitis

Table 2 Clinical outcomes according to phase angle classifications, *n* (%)

Variable	*n* = 129	< 5.4, *n* = 89	> 5.4, *n* = 40	< 50, *n* = 56	> 50, *n* = 73	mean ± SD, PA
Deaths from the 1st year to the 5th year (1-60 mo)	41	17 (41.46)	24 (58.53)	22 (53.65)	18 (46.35)	5.81 ± 1.87
Deaths from the 6th to the 10th year (61-120 mo)	19	7 (36.84)	12 (63.15)	10 (52.63)	9 (47.36)	6.63 ± 3.21
Deaths from the 11th to the 15th year (121-180 mo)	6	1 (16.67)	5 (83.33)	2 (33.34)	4 (66.66)	6.65 ± 1.90
Alive not transplanted	45	6 (13.33)	39 (86.66)	11 (24.44)	34 (75.55)	7.04 ± 3.07
Alive transplanted	10					8.62 ± 5.48
Dead transplanted	8	4 (50)	4 (50)	6 (75)	2 (25)	5.75 ± 1.01
Deaths from the 1st year to the 5th year (1-60 mo)	3	1 (33.33)	2 (66.67)	1 (33.33)	2 (66.67)	6.57 ± 1
Deaths from the 6th to the 10th year (61-120 mo)	4	2 (50)	2 (50)	4 (100)	-	5.28 ± 0.56
Deaths from the 11th to the 15th year (121-180 mo)	1	1 (100)	0	1 (100)	-	5.19 ± 0

Results expressed by mean ± SD, median ± SD or *n* (%).

The mortality rate of the non-transplanted patients (*n* = 111) over the 15-year period showed that 66 died (59.45%), and among these, 41 (62.12%) died within the first to fifth year, 19 (28.78%) from the sixth to the tenth year, and 6 (9.09%) from the eleventh to the fifteenth year. Of the 41 who died in the first five years, 41.46% had a PA < 5.4° and 53.65% had a percentile < P50. Of the 19 who died between the sixth and tenth year, 36.84% had PA< 5.4, and 52.63% had < P50. The 6 patients who died in the last 5 years of the study, 16.66% had PA < 5.4 and 33.33% had < P50 (Table 2).

Table 3 Clinical outcomes according to phase angle classifications, %

Variables	Total, *n* = 129	< P50, *n* = 56	> P50, *n* = 73
Deaths	57.4	71.40	46.60
SPB	18.6	23.2	15
Ascites	57	66.1	50.70
Paracentesis	30.20	32.10	28.80
EV	75	82.10	69.90
Varicose veins bleeding	10	10.71	10.95
Encephalopathy	34.9	46.40	26
HCC	8.50	10.70	6.80

SPB: Spontaneous bacterial peritonitis; EV: Esophageal varices; HCC: Hepatocellular carcinoma.

Table 4 Biomarkers according to percentil classification

Variable	*P* < 50, *n* = 56	*P* > 50, *n* = 73	*P* value
GOT (mg/dL) mean ± Dp	128 ± 428	71 ± 47	0.27
GPT (mg/dL) mean ± Dp	79 ± 227	58 ± 42	0.46
GGT (mg/dL) mean ± Dp	102 ± 80	94 ± 103	0.69
ALP (mg/dL) mean ± Dp	117.4 ± 55.1	101.4 ± 54.6	0.13
Albumin (g/dL) mean ± Dp	3.6 ± 1.9	3.9 ± 0.6	0.34
Total Bilirubin (mg/dL) mean ± Dp	2.4 ± 2.6	1.7 ± 1.2	**0.02**[a]
Direct Bilirubin (mg/dL) mean ± SD	1 ± 1.1	0.7 ± 0.5	**0.02**[a]
Indirect bilirubin (mg/dL) mean ± SD	1.3 ± 1.5	0.9 ± 0.9	0.10
Urea (mg/dL) mean ± SD	36 ± 16	36 ± 13	0.79
Creatinine (mg/dL) mean ± SD	1 ± 0.3	1 ± 0.2	0.64
Sodium (mg/dL) mean ± SD	137 ± 4	139 ± 4	**0.03**[a]
Potassium (mg/dL) mean ± SD	4.6 ± 2	4.9 ± 2.7	0.32
INR mean ± SD	1.36 ± 0.38	1.45 ± 0.51	**0.02**[a]

[a]T test for independent samples.
GOT: Oxaloacetic Transaminase; GPT: Pyruvic Transaminase; ALP: Alkaline phosphatase; INR: International normalized ratio.

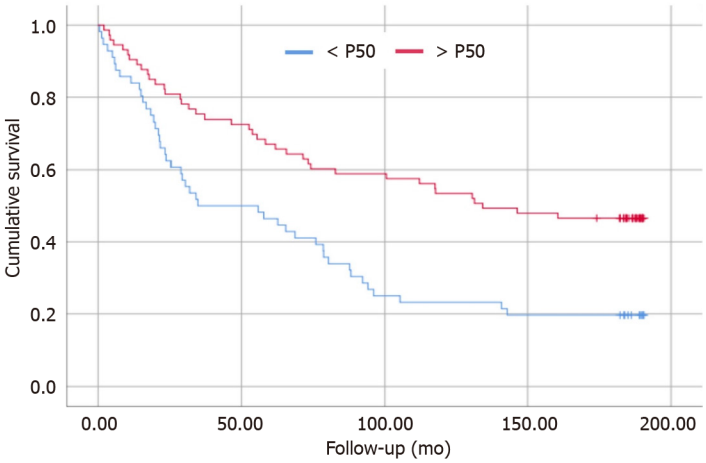
Over the 15 years, 18 patients underwent liver transplantation, and 8 (44.44%) died. Of these, 4 (50%) died in the first six years and the remaining 50% between the seventh and fifteenth year. Of the patients who died between the first and sixth year, 2 (50%) had P50 and a PA 5.40, and those who died between the seventh and fifteenth years, 4 (100%) had P50, and 50% had a PA 5.40.

The average age of the transplanted patients was 51 (SD 51 ± 11) years, 61.11% were male. Among the main causes of transplantation, we cite C virus (27.77%) and C virus + alcohol (16.66%). The leading cause of death of the transplanted patients was post-transplant infection (37.5%). When analyzing the cause of death according to the percentile, infection was the predominant cause (50%) in the < P50 group, while in the >P50 group, the unknown cause and pulmonary cause had the same distribution (50%).

Upon analyzing the clinical outcomes, 57.40% of the patients died due to cirrhosis. Among them, 71.4% had a PA in the $P < 50$. As for cirrhosis complications, 57% of the patients had ascites, 34% developed encephalopathy, and 75% had esophageal varices (EV), of which 10.9% presented with upper gastrointestinal bleeding (Table 3).

Patients classified between the P25 and P50 had a higher occurrence of EV; however, those classified between the P5 and P25 had a higher occurrence of variceal bleeding as well as encephalopathy.

The serum values of total bilirubin and direct bilirubin were significantly higher in the $P < 50$ group ($P = 0.02$) in addition, this same group had significantly lower levels of sodium ($P = 0.03$) and INR ($P = 0.02$) as shown in Table 4.



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Figure 1 Survival curves.

Upon verifying the relationship between the PA and the duration of hospitalization, the interval in days between hospitalizations, and the survival time, it was observed that the phase angle is significantly correlated with survival time (R^2 = 0.242; P = 0.006), and the length of hospital stay (Rho = -0.201; P = 0.02).

The survival curve is a point that deserves attention regarding the PA values reflecting the staging of cirrhosis. Patients with PA < P50 presented higher mortality when compared to patients in the > P50 group (Figure 1). Through logistic regression, for each 1° increase in PA, the patient increases their chance of survival by 17.7%.

DISCUSSION

The natural history of cirrhotic patients indicates an average survival duration of approximately 10 years. However, depending on their clinical conditions, some patients may exhibit distinctive cirrhosis complications that negatively impact morbidity and mortality[12-14].

Early diagnosis or treatment of these complications prolongs survival time, improves the quality of life for cirrhotic patients, and reduces the mortality rate of patients on the waitlist for liver transplantation.

Accordingly, this study evaluated 129 cirrhotic patients over a 15-year follow-up to identify a non-invasive, practical, observer-independent, and easily reproducible parameter capable of predicting cellular impairment in the patho-physiological evolution of cirrhosis.

The demographic characteristics of the population in this study align with the findings of Belarmino's study, which followed 134 cirrhotic patients with an average age of 54.3 (SD 54.3 ± 10.10), predominantly male[4]. Similar demographic findings were noted in Ruiz *et al*'s cohort[15], which assessed 136 cirrhotic patients, again predominantly male, with an average age of 54.5 (SD 54.5 ± 10.0). However, it is noteworthy that the population of this study presented compensated cirrhosis, as opposed to the findings by Belarmino *et al*[4], where only 18% were classified as Child-Pugh A, 55% as B, and 27% as C, and the average MELD score was 14.11 (SD14.11 ± 4,95). Ruiz *et al*'s study included a homogeneous sample according to the Child-Pugh classification, with 34.1% B, 33.3% C, and a MELD score of 14 (14 ± 6)[15].

Regarding mortality, the increased percentage of deaths is related to a reduction in PA in degrees and percentile. These findings align with Belarmino *et al*[4] study, which identified that patients with PA less than 4.9°, even with compensated cirrhosis, had a higher mortality rate. A similar mortality outcome associated with PA was observed in Saueressig *et al*[16] study, evaluating 97 hospitalized cirrhotic patients with decompensated disease throughout 11.2 mo (2.4-21). However, it is worth highlighting that the same study established 5.52° as the cut-off point for PA, resembling the 5.4° value utilized in this study, but with divergent disease staging characteristics as per the Child-Pugh score, being 9.3% A, 60.8% B, and 29.9% C. This strengthens the hypothesis that PA reflects systemic cellular damage, not solely linked to the parameters used for score calculations.

Regarding liver transplantation, the etiological presence of hepatitis C virus was observed, recalling that data collection began in 2007. Until 2014, hepatitis C cirrhosis was the leading cause of liver transplantation. However, a study conducted between 2014 and 2019, including 51329 patients listed for transplantation, demonstrated a change in this scenario due to an increased incidence of MASLD and alcoholic cirrhosis, justified by rising obesity levels and an increase in alcoholic individuals[17].

Serrano *et al*[18] study, evaluating the outcome of 15998 liver transplants over 10 years, sustains the findings of this study, showing an exponential increase in the mortality rate. On the other hand, the authors observed a difference in survival between men and women, as in the first year, male mortality was higher, which did not occur in subsequent years. This difference in findings between the two studies can be justified by the size of the population studied by Serrano *et al*[18].

Nitski *et al*[19] evaluated 42146 Liver transplant recipients from the Scientific Registry of Transplant Recipients in the US (UHN) and data from the University Health Network (UHN) in Canada, with average follow-ups of 8 and 5 years, respectively, and found that among the leading causes of death in post-liver transplant patients were various types of cancer, infection, organ rejection, and cardiovascular causes, including stroke. These findings align with the results found in this study, which shows a predominance of deaths due to infection followed by HCC and stroke. Patients who died due to infection had < P50, demonstrating the potential of PA as a predictive value of cellular impairment related to the inflammatory process[20,21].

Ascites, encephalopathy, and EV are commonly observed complications in the cirrhosis picture. Román *et al*[22], evaluating 100 cirrhotic patients, described that 63% of these had ascites, 12% had encephalopathy, and 31% had EV, unlike the findings of this study, which showed a prevalence of encephalopathy and EV. The difference in disease staging could explain the difference between the studies, as in Román *et al*'s study, 81% of patients were classified as Child-Pugh A[23].

Shi *et al*[24] evaluated 248 cirrhotic patients over 4 years, with the variables analyzed including serum levels of AST, GGT, AP, bilirubin, albumin, and PA. The authors presented a statistically significant correlation of patients with PA < 5.1° (the cut-off point for this patient sample) with increased levels of albumin, total and direct bilirubin, which corroborates this study.

Hospitalization costs for cirrhotic patients grew by 30.2% from 2008 to 2014. Concurrently, there was a 36% increase in hospitalizations of compensated cirrhotic patients and a 24% gain in those of decompensated patients. Hospitalizations of patients with decompensated cirrhosis represented 58.6% of the total hospitalizations of cirrhotic patients in 2014. The main operators of rising costs are the costlier procedures, escalating from 15% to 152%, and the presence of clinical complications[25].

Román *et al*[22] observed that PA is also related to the occurrence of clinically relevant events. The same was observed in this study, where a significant correlation was found between PA, the number of hospitalizations (P = 0.01), length of hospital stay (P = 0.003), and mortality (P = 0.001).

The survival curve presented by various studies[7,26-29] evaluating PA in cirrhotic patients reinforces the results found in this study, demonstrating that a reduction in PA, regardless of the cut-off point, is associated with a growth in morbidity and mortality of cirrhotic patients.

Among the study's limitations, we mention the manual recording of collected data in the patient's medical record, which makes detailing some information impracticable, such as the degree of cirrhosis complications. However, the outcome of the 129 patients included in the study was described.

CONCLUSION

This study is a pioneer on PA as a predictor of mortality in cirrhotic patients, with PA values per percentile for the Brazilian population. The PA by percentile showed greater sensitivity in predicting mortality compared to the cut-off point of 5.4°.

The PA, measured through electrical bioimpedance, can be measured in a segmented way, where the patient can be evaluated daily. In more specific cases, such as in the case of ascites, the measurement of the PA can be performed before and after the paracentesis, informing the cellular condition after a procedure. Thus, the PA becomes a guiding tool in the clinical management of cirrhotic patients by significantly reducing the number of events due to complications characteristic of chronic liver disease. The use of PA can bring as positive outcomes: a lower number and length of hospitalizations, improved quality of life, better results in liver transplantation and, consequently, increased survival time.

ARTICLE HIGHLIGHTS

Research background

The number of new cases of cirrhotic patients is growing worldwide and, as a consequence, an increase in the demand for specialized care to treat the disease *per se* and the complications inherent in cirrhosis, in addition to the increase in patients on the waiting list for orthotopic transplantation of liver. Given this scenario, it is necessary to identify a tool to predict the mortality of these patients linked to their clinical condition. Within this perspective, the phase angle (PA) becomes a good alternative because it is a viable method in clinical practice, with the potential to guide the clinical management of the patient and extend their survival time and better quality of life.

Research motivation

Identifying a tool capable of predicting mortality and severity of chronic liver disease in real time as a clinical practice brings numerous benefits to this population and to the professionals who treat them.

Research objectives

To the best of our knowledge, no study has evaluated the role of PA as a predictor of mortality in cirrhotic patients with a 15-year follow-up.

Research methods

Retrospective cohort study with 129 cirrhotic patients of both genders aged over 18 years. Diagnosis of cirrhosis by liver biopsy. The cut-off value for the PA was 5.4°, a value described in 2012 by Fernandes *et al* for 129 patients evaluated in this study and the cut-off points for the Brazilian population presented in percentiles (P), as described by Mattiello *et al*. Mortality was assessed using the PA percentile using Kaplan-Meier curves and multivariate binary logistic regression models.

Research results

The percentile ranking was more accurate in identifying long-term deaths than the 5.4th PA. Patients with < P50 had a higher number of relevant complications, such as ascites, SBP, liver encephalopathy and HCC. PA is strongly correlated with serum albumin ($P < 0.001$), INR ($P = 0.01$), total bilirubin ($P = 0.02$) and direct bilirubin ($p = 0.003$). PA is correlated with survival time ($P < 0.001$) and length of stay ($P = 0.02$). Logistic regression analysis shows that a 1° increase in PA increases the chance of survival of cirrhotic patients by 17.7%.

Research conclusions

PA is a good predictor of morbidity and mortality for cirrhotic patients.

Research perspectives

Identifying clinical factors that enhance a poor prognosis for cirrhotic patients, such as ascites, encephalopathy, length of stay, is relevant. With this information, it is possible to act early in the clinical management of these patients and increase the effectiveness of the therapeutic response, with consequent improvement in the prognosis and quality of life of this population.

FOOTNOTES

Author contributions: Pinto LP contributed to the methodological development and material support, collected data, interpreted results, conducted data analysis, wrote and revised the manuscript; Marroni AC contributed to the conception and critical review of the manuscript; Czermainski J collected data and interpreted the results; Dahlem MLF contributed to the methodological development and material support; Carteri R conducted data analysis and interpreted the results; Fernandes SA designed the research project, collaborated in writing, and critically reviewed the manuscript; All authors have read and approved the final manuscript.

Institutional review board statement: This study was conducted in accordance with the Helsinki Declaration and was approved by the ethics and research committee of the Federal University of Health Sciences of Porto Alegre (UFCSPA), RS, Brazil, under the number 5203619. All participants signed the Informed Consent Form (TCLE) in advance.

Informed consent statement: All study participants or their legal guardian provided informed written consent about personal and medical data collection prior to study enrolment.

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Country/Territory of origin: Brazil

ORCID number: Letícia Pereira Pinto 0000-0001-8543-1587; Claudio Augusto Marroni 0000-0002-1718-6548; Juliana Czermainski 0000-0002-4628-2467; Maria Luiza Fernandes Dahlem 0009-0009-6088-0074; Randhall B Carteri 0000-0003-4124-9470; Sabrina Alves Fernandes 0000-0001-8504-603X.

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ORIGINAL ARTICLE

Retrospective Study

Association of carbon monoxide poisonings and carboxyhemoglobin levels with COVID-19 and clinical severity

Abuzer Coskun, Burak Demirci, Kenan Ahmet Turkdogan

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Abuzer Coskun, Burak Demirci, Emergency Medicine Clinic, Istanbul Bagcilar Training and Research Hospital, Istanbul 34200, Turkey

Kenan Ahmet Turkdogan, Emergency Medicine Department, Istanbul Çam and Sakura City Hospital, Istanbul 34494, Turkey

Corresponding author: Abuzer Coskun, MD, Associate Professor, Emergency Medicine Clinic, Istanbul Bagcilar Training and Research Hospital, No. 2, Dr. Sadik Ahmet Street, Istanbul 34200, Turkey. dr.acoskun44@hotmail.com

Abstract

BACKGROUND

Coronavirus disease 2019 (COVID-19), which recently spread throughout the entire world, is still a significant health issue. Additionally, the most common cause of risky poisoning in emergency services is carbon monoxide (CO) poisoning. Both disorders seem to merit more research as they have an impact on all bodily systems *via* the lungs.

AIM

To determine how arterial blood gas and carboxyhemoglobin (COHb) levels affect the clinical and prognostic results of individuals requiring emergency treatment who have both COVID-19 and CO poisoning.

METHODS

Between January 2018 and December 2021, 479 CO-poisoning patients participated in this single-center, retrospective study. Patients were primarily divided into two groups for analysis: Pre-pandemic and pandemic periods. Additionally, the pandemic era was divided into categories based on the presence of COVID-19 and, if present, the clinical severity of the infection. The hospital information system was used to extract patient demographic, clinical, arterial blood gas, COVID-19 polymerase chain reaction, and other laboratory data.

RESULTS

The mean age of the 479 patients was 54.93 ± 11.51 years, and 187 (39%) were female. 226 (47%) patients were in the pandemic group and 143 (30%) of them had a history of COVID-19. While the mean potential of hydrogen (pH) in arterial blood gas of all patients was 7.28 ± 0.15, it was 7.35 ± 0.10 in the pre-pandemic group and 7.05 ± 0.16 in the severe group during the pandemic period ($P < 0.001$).

COHb was 23.98 ± 4.19% in the outpatients and 45.26% ± 3.19% in the mortality group ($P < 0.001$). Partial arterial oxygen pressure (PaO_2) was 89.63 ± 7.62 mmHg in the pre-pandemic group, and 79.50 ± 7.18 mmHg in the severe group during the pandemic period ($P < 0.001$). Despite the fact that mortality occurred in 35 (7%) of all cases, pandemic cases accounted for 30 of these deaths (85.7%) ($P < 0.001$). The association between COHb, troponin, lactate, partial arterial pressure of carbon dioxide, HCO_3, calcium, glucose, age, pH, PaO_2, potassium, sodium, and base excess levels in the pre-pandemic and pandemic groups was statistically significant in univariate linear analysis.

CONCLUSION

Air exchange barrier disruption caused by COVID-19 may have pulmonary consequences. In patients with a history of pandemic COVID-19, clinical results and survival are considerably unfavorable in cases of CO poisoning.

Key Words: Emergency department; Coronavirus disease 2019; Carbon monoxide; Mortality; Carboxyhemoglobin; Intoxication; Poisoning

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Core Tip: This retrospective study included 479 patients with a mean age of 54 years. The association of both coronavirus disease 2019 and carbon monoxide poisoning in the emergency department has not been described in the literature. This study includes meticulous work on this association carried out in the emergency room. The clinical, hospitalization, complication and mortality rates were evaluated.

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INTRODUCTION

Carbon monoxide (CO) is an odorless, tasteless, nonirritating gas produced by the incomplete combustion of carbon compounds. It has been reported as one of the most prevalent causes of death, accounting for 31% of toxic poisonings[1]. CO is the third leading cause of accidental gas inhalation-related death in the United States[2]. This gas is readily absorbed and unaltered by the lungs. 90% is bound to hemoglobin (Hb), 10% to myoglobin, and 10% to cytochrome C-oxidase after absorption. Less than 1% is dissolved in plasma, and less than 1% is oxidized to carbon dioxide[3]. CO binds with high affinity to Hb in the blood to form carboxyhemoglobin (COHb). Exposure to CO levels as low as 10 ppm can result in approximately 2% COHb[4]. CO is 250 times more attractive than oxygen to Hb. CO competes with oxygen for binding to Hb, reducing oxygen transport capacity[5,6]. CO prevents oxidative phosphorylation by inhibiting mitochondrial respiration *via* heme a3 binding. As a result, it causes hypoxia in many organs[7].

Signs of lung injury in coronavirus disease 2019 (COVID-19) can range from minimal to severe acute respiratory distress syndrome (ARDS)[8]. Silent hypoxemia is the most important factor in COVID-19 patients. This term refers to arterial hypoxemia in patients who are conscious and alert but have no significant dyspnea. In certain instances, there is profound hypoxemia with pulse oximetry values of 70% and partial arterial oxygen pressure (PaO_2) values of 40 mmHg [9]. Associated with the phenomenon of silent hypoxemia are parenchymal compliance, hypoxic pulmonary vascularity, ventilation control, and dyspnea. The causes of hypoxemia directly initiate inflammation *via* viral infection and secondary immune response. Disease progression can result in diffuse alveolar damage, exudative-proliferative stages, hyaline membrane structure damage, edema, atypical pneumocyte hyperplasia, alveolar hemorrhage, endothelial cell damage, micro-thrombosis, dilatation, and characteristic ARDS features, including hypoxemia due to capillary occlusion[10,11]. Vascular findings, which also occur in many other organs, have led to the belief that COVID-19 patients experience lung injury and significant hypoxia[12]. Although CO poisoning and COVID-19 cause hypoxia *via* distinct mechanisms, it is evident that they interact to reduce lung diffusion capacity. Mo *et al*[13] examined the conventional lung capacity of mild, moderate, and severe COVID-19 survivors 20–30 d after the onset of symptoms. Despite relatively normal spirometry, patients had a 50% reduction in lung diffusion CO capacity (DLCO) and a 25% reduction in DLCO/alveolar volume. In his study, Nusair[14] found that low DLCO is primarily attributable to decreased alveolar volume, and not residual interstitial or pulmonary vascular abnormalities caused by COVID-19.

In the present study, we aimed to determine the cumulative increasing mortality rates, the effects of high COHb levels, and serum lactate levels in COVID-19 patients who were exposed to CO poisoning during the pre-pandemic and pandemic periods.

MATERIALS AND METHODS

Study design and population

This cross-sectional cohort analysis comprised 479 patients over the age of 18 years with CO poisoning who attended the emergency department between January 2018 and December 2021. All CO poisonings were caused by heating system malfunctions or accidents. Our hospital's registration system includes patient diagnoses, admission dates, contact information, and demographic, clinical, and laboratory data. Furthermore, pre-pandemic and pandemic CO poisoning cases, pandemic COVID-19 cases, patient polymerase chain reaction (PCR) records, and data from patients who presented to our hospital with CO poisoning are all incorporated in our system.

When categorizing the patients, two groups were included: Pre-pandemic and pandemic. Patients between January 2018 and December 2019 were classified as pre-pandemic, whereas those between January 2020 and December 2021 were classified as pandemic. Patients in the pre-pandemic group were chosen by evaluating individuals who presented to the emergency clinic due to CO poisoning and had a COHb value greater than 10%. Both non-COVID-19 CO poisoning cases and cases with COVID-19, or those with positive PCR results and exposure to CO poisoning, were included in the pandemic group, as long as the COHb value was greater than 10%. The study comprised patients whose arterial blood gas, serum lactate, troponin I value, and CO exposure periods were known and recorded at the time of admission to the emergency department.

Patients with a coma score of less than 10, prior cerebrovascular disease, significant psychiatric illness or drug use, a history of infectious disease other than COVID-19, and pregnant patients were excluded from the study. Furthermore, individuals less than 18 years old with unknown arterial blood gas, troponin I, serum lactate levels, or CO exposure duration were excluded from the study.

Cases exposed to acute CO poisoning during the pre-pandemic and pandemic eras were divided into two groups. The pandemic period was also divided into two parts: Non-COVID-19 and COVID-19. Those who tested positive or had COVID-19 were also evaluated according to their clinical condition, which was divided into three categories: Mild, moderate, and severe[15]. Four groups were constructed based on the clinical history of the patients: Outpatient follow-up, hospitalization, intensive care unit (ICU), and mortality. All patients who died in the emergency department or in the critical care unit died during the acute period. The approximate CO inhalation time was used for determining the CO exposure time.

Laboratory analysis

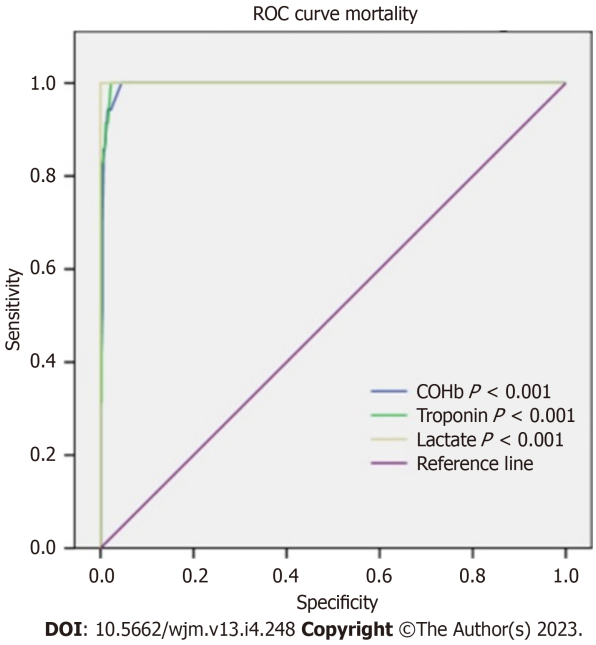
The patients' COHb levels were assessed by arterial blood gas analyses performed using ABL 835 Flex Radiometer laboratory instruments, Blood Gas system (Aknlab, Istanbul, Turkey). Arterial blood gas data were examined in 5-10 min, and individuals with a COHb value of 10% or higher were classified as having CO poisoning and participated in the study. As clinical results were not detected, COHb values ranging from 1% to 10% were excluded from the study. Depending on their clinical status, all patients with COHb levels above 15% received hyperbaric oxygen therapy for 1-3 sessions. Serum lactate levels were also measured during arterial blood gas analysis, and values between 0.5 and 1.6 mmol/L over the reference range were considered significant. Troponin I STAT Elecsys and Cobas e 411 Hitachi (Roche, Swaziland) analyzers were used to measure Troponin I levels. Troponin I results were analyzed between 45-60 min and levels above 0.05 ng/mL were considered significant.

Statistical analysis

The SPSS 20.0 software package (SPSS Inc., Chicago, IL, United States) was used to analyze the data in this study. The normal distribution of the variables was examined using a one-sample Kolmogorov-Smirnov test. As the variables did not have a normal distribution, the Kruskal-Wallis-H test was used to compare the groups. The associations between nominal variable groups were investigated using Chi-square analysis. Spearman's correlation analysis was used to determine the correlation between groups. Furthermore, linear regression was employed to identify univariate and multivariate variable analyses. Univariate analysis was used to determine the association between patient groups and factors. Univariate analysis factors that were statistically significant were used in the multivariate linear regression risk model. The sensitivity and specificity of the COHb, troponin, and lactate mortality values were evaluated using a Receiver Operating Characteristic curve. $P < 0.05$ was declared statistically significant for interpreting the results.

RESULTS

The mean age of the 479 patients was 54.93 ± 11.51 years, 187 (39%) were female, and the age range was 23-78 years. The mean age in the pre-pandemic group was 50.56 ± 11.24 years, and was 60.76 ± 8.44 years in the pandemic group ($P < 0.001$). The relationship between the pre-pandemic/pandemic period and gender was not statistically significant. The duration of exposure to CO poisoning in all patients was 4.31 ± 1.74 h, and there was no significant difference between the groups ($P = 0.201$). When the arterial blood gases of all patients were evaluated, the mean potential of hydrogen (pH) was 7.28 ± 0.15, while it was 7.35 ± 0.10 in the pre-pandemic group and 7.05 ± 0.16 in the severe group during the pandemic period ($P < 0.001$). In addition, while the partial arterial pressure of carbon dioxide ($PaCO_2$) was 43.32 ± 6.81 mmHg in the pre-pandemic group, it increased to 57.76 ± 4.49 mmHg in the pandemic group ($P < 0.001$). Likewise, while PaO_2 was 89.63 ± 7.62 mmHg in the pre-pandemic group, this value decreased to 79.50 ± 7.18 mmHg in the severe group ($P < 0.001$). Mean potassium level was 4.20 ± 0.71 mmol/L ($P < 0.001$), sodium was 138.04 ± 4.52 mmol/L ($P = 0.239$), and calcium was 1.16 ± 0.26 mmol/L ($P = 0.020$). It was observed that the glucose level, which was 120.49 ± 20.98 mg/dL in



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Figure 1 Receiver operating characteristic curve for mortality. ROC: Receiver operating characteristic.

the pre-pandemic group, increased to 154.50 ± 15.73 mg/dL in the pandemic group (P < 0.001). In addition, while bicarbonate (HCO_3) was 20.31 ± 5.46 mmol/L in the pre-pandemic group, it decreased to 9.92 ± 3.73 mmol/L, which was the lowest level in the pandemic group. The mean base deficit was 7.09 ± 5.56 mmol/L (P < 0.001). While lactate was in the normal range at 1.89 ± 1.24 mmol/L in the pre-pandemic group, it increased to 6.33 ± 1.76 mmol/L in the pandemic group (P < 0.001). COHb value was 26.19% ± 6.68% in the pre-pandemic group and 41.08% ± 7.55% in the pandemic group (P < 0.001). Troponin I value was 0.11 ± 0.25 ng/mL in the pre-pandemic group and 1.09 ± 0.50 ng/mL in the pandemic group (P < 0.001) (Table 1).

In the analysis according to the survival status of the patients, the mean age of the outpatients was 50.27 ± 10.67 years, while the mean age of the patients who died was 66.63 ± 6.95 years (P < 0.001). There was no significant relationship between survival and gender. The duration of exposure to CO poisoning was determined as 5.14 ± 1.78 h in the mortality group (P < 0.001). In addition, COHb was 23.98% ± 4.19% in the outpatients and 45.26% ± 3.19% in the mortality group (P < 0.001). Troponin I was found to be increased at 1.35 ± 0.36 ng/mL and lactate at 8.14 ± 0.63 mmol/L in the mortality group (P < 0.001). In addition, in the analysis of the patient groups by survival, it was seen that 10 (4%) patients in the pre-pandemic group were in the ICU and 5 (2%) of these patients were in the mortality group. Fifty-nine (26.1%) of 226 patients in the pandemic group were followed in the ICU, and 30 (13.3%) died (P < 0.001) (Table 2).

In the univariate linear analysis, COHb, troponin, lactate, $PaCO_2$, HCO_3, calcium, glucose, age, pH, PaO_2, potassium, sodium, and base excess levels were found to be statistically significant in the pre-pandemic and pandemic groups. On the other hand, in multivariate linear regression analysis, COHb, troponin, lactate, $PaCO_2$, HCO_3, calcium, and glucose values were found to be prognostic signs in the pre-pandemic and pandemic groups (Table 3).

Changes in COHb, lactate, and troponin due to CO poisoning are shown in Figure 1 based on the receiver operating characteristic curve analysis. Based on this analysis, the optimal cut-off values (sensitivity and specificity), the area under the curve, and the 95% confidence interval of COHb, lactate, and troponin were found to be over 45% to predict the evolution of the pre-pandemic and pandemic groups (P < 0.001). In addition, in the correlation analysis of the variables for patient groups and survival, a medium-strong positive relationship was found between age, exposure time, COHb, troponin, lactate, $PaCO_2$, glucose, and base excess, and a strong negative relationship with HCO_3, pH, and PaO_2 (Tables 4 and 5).

DISCUSSION

CO intoxication and COVID-19 are both serious disorders that impact the respiratory system, impairing oxygenation and causing hypoxia. There are numerous studies on CO poisoning and COVID-19 in the literature. However, in our search of the literature, we did not discover any studies in which both diseases coexisted. This encouraged us to explore the morbidity and mortality effects of CO poisoning in patients with current or previous COVID-19. We found that the mortality rate during the pandemic period, including hyperbaric oxygen, mechanical ventilation, and all ICU treatments, was 6.4 times greater in COVID-19 patients with a COHb value of 10% or higher than the mortality rate before the pandemic.

Although the pathophysiological basis of CO poisoning is not clear, recent studies suggest that different mechanisms play a role in the toxicity caused by CO[16-18]. CO combines with respiratory pigments, enzymes, and proteins

Table 1 Basal and laboratory findings of the patients

			Disease periods					
				Pandemic (*n* = 226)				
		All patients (*n* = 479), mean ± SD	Pre-pandemic, (*n* = 253), mean ± SD	Non-COVID-19 (*n* = 83), mean ± SD	Mild COVID-19 (*n* = 60), mean ± SD	Moderate COVID-19 (*n* = 45), mean ± SD	Severe COVID-19 (*n* = 38), mean ± SD	*P* value
Baseline characteristics								
Age (year)		54.93 ± 11.51	50.56 ± 11.24	56.87 ± 10.29	57.32 ± 9.58	65.98 ± 6.70	62.87 ± 7.50	**< 0.001**
Gender	Female	187 (39)	98 (38.7)	38 (45.8)	24 (40)	16 (35.6)	11 (28.9)	0.3461
	Male	292 (61)	155 (61.3)	45 (54.2)	36 (60)	29 (64.4)	27 (71.1)	
Exposure time (h)		4.31 ± 1.74	4.09 ± 1.86	4.84 ± 1.98	4.25 ± 1.26	4.69 ± 1.24	4.24 ± 1.23	0.201
Laboratory findings								
Arterial blood gas	pH (7.35-7.45)	7.28 ± 0.15	7.35 ± 0.10	7.29 ± 0.12	7.23 ± 0.12	7.14 ± 0.16	7.05 ± 0.16	**< 0.001**
	$PaCO_2$ (mmHg) (32-45)	47.07 ± 8.34	43.32 ± 6.81	46.04 ± 7.90	51.10 ± 6.34	55.67 ± 4.93	57.76 ± 4.49	**< 0.001**
	PaO_2 (mmHg) (80-100)	86.92 ± 8.01	89.63 ± 7.62	87.10 ± 6.44	83.01 ± 7.40	82.81 ± 6.50	79.50 ± 7.18	**< 0.001**
	K+ (mmol/L) (3.4-4.5)	4.20 ± 0.71	4.00 ± 0.58	4.01 ± 0.55	4.34 ± 0.78	4.64 ± 0.64	5.25 ± 0.63	**< 0.001**
	Na+ (mmol/L) (135-149)	138.04 ± 4.52	138.52 ± 4.70	137.35 ± 4.33	137.98 ± 4.22	17.13 ± 4.23	137.48 ± 4.33	0.239
	Ca++ (mmol/L) (1.15-.29)	1.16 ± 0.26	1.18 ± 0.25	1.19 ± 0.26	1.11 ± 0.20	1.07 ± 0.29	1.06 ± 0.37	**0.02**
	Cl- (mmol/L) (98-106)	99.90 ± 8.27	99.97 ± 7.14	100.01 ± 5.72	100.01 ± 12.69	99.98 ± 10.19	98.92 ± 9.05	0.944
	BS (mg/Dl) (70-105)	129.59 ± 24.66	120.49 ± 20.98	129.35 ± 21.89	132.75 ± 24.18	155.91 ± 21.58	154.50 ± 15.73	**< 0.001**
	HCO_3 (mmol/L) (22-26)	17.68 ± 6.18	20.31 ± 5.46	17.91 ± 5.49	15.13 ± 4.59	12.47 ± 4.62	9.92 ± 3.73	**< 0.001**
	BE (mmol/L) (-3.0-3.0)	7.09 ± 5.56	4.76 ± 4.68	6.76 ± 4.85	9.22 ± 4.43	11.79 ± 4.48	14.41 ± 3.58	**< 0.001**
	Lactate (mmol/L) (0.5-1.6)	2.85 ± 1.99	1.89 ± 1.24	2.60 ± 1.57	3.54 ± 1.44	4.87 ± 1.92	6.33 ± 1.76	**< 0.001**
	COHb (%) (0.5-1.5)	29.68 ± 7.85	26.19 ± 6.68	27.63 ± 5.37	34.25 ± 5.00	37.35 ± 5.38	41.08 ± 7.55	**< 0.001**
Troponin I (ng/mL) (0.0-0.05)		0.40 ± 1.91	0.11 ± 0.25	0.20 ± 0.28	0.42 ± 0.35	0.82 ± 0.31	1.09 ± 0.50	**< 0.001**

[1]Chi-Square test.

Other *P* values were calculated by the Kruskal-Wallis H test. Bold values indicate significance at $P < 0.05$. SD: Standard Deviation; COVID-19: Coronavirus disease 2019; pH: Potential of Hydrogen; $PaCO_2$: Partial pressure of carbon dioxide; PaO_2: Partial arterial oxygen pressure; K^+: Potassium; Na^+: Sodium; Ca^{++}: Calcium; Cl^-: Chloride; BS: Blood sugar; HCO_3: Bicarbonate; BE: Base Excess; COHb: Carboxyhemoglobin.

(myoglobin, Hb, cytochrome a3, and cytochrome P450). It is thought to act as a result of binding with cytochrome oxidase enzymes, such as cytochrome-a3, which is the terminal enzyme in the electron transport chain. Hypoxia and decreased blood flow cause CO to bind and inhibit cytochrome C oxidase and impair cellular respiration at the mitochondrial level [19,20]. CO, at toxic levels, activates platelets by increasing the frequency of thrombosis. It then stimulates neutrophils, leading to myeloperoxidase release, generation of reactive oxygen species, and inflammation[21]. As a result of these, aerobic respiration is affected, adenosine triphosphate production is disrupted, and if progression of this process is not prevented, lactic acidosis accumulates in the cells and death occurs when the cells begin anaerobic respiration. In

Table 2 Analysis of patient survival, baseline values and variables (mean ± SD)

Survival			Outpatient (*n* = 258)	Hospitalization (*n* = 117)	ICU (*n* = 69)	Mortality (*n* = 35)	*P* value
Baseline characteristics							
Age (yr)			50.27 ± 10.67	47.76 ± 11.05	61.61 ± 7.56		**< 0.001**
Gender		Female	107 (41.5)	47 (40.2)	21 (30.4)	12 (34.3)	0.128[1]
		Male	151 (58.5)	70 (59.8)	48 (69.6)	23 (65.7)	
Exposure time (h)			3.90 ± 1.62	4.45 ± 1.68	5.19 ± 1.79	5.14 ± 1.78	**< 0.001**
COHb (%)			23.98 ± 4.19	3238 ± 2.81	38.49 ± 2.99	45.26 ± 3.19	**< 0.001**
Troponin I (ng/mL)			0.04 ± 0.09	0.33 ± 0.27	0.77 ± 0.21	1.35 ± 0.36	**< 0.001**
Lactate (mmol/L)			1.42 ± 0.39	3.54 ± 0.95	4.34 ± 0.68	8.14 ± 0.63	**< 0.001**
Patient groups	Pre-pandemic		198 (76.7)	40 (34.2)	10 (14.5)	5 (14.3)	**< 0.001**[1]
	Pandemic	Non-COVID-19	50 (19.4)	26 (22.2)	5 (7.2)	2 (5.7)	
		Mild	8 (3.1)	28 (23.9)	21 (30.4)	3 (8.6)	
		Moderate	2 (0.8)	16 (13.7)	17 (24.6)	10 (28.6)	
		Severe	0	7 (6)	16 (23.2)	15 (42.9)	

[1]Chi-Square test.

Other *P* values were calculated by the Kruskal-Wallis H test. Bold values indicate significance at $P < 0.05$. SD: Standard deviation; COVID-19: Coronavirus disease 2019; ICU: Intensive care unit; COHb: Carboxyhemoglobin.

autopsies, the lungs in CO poisonings are swollen, edematous, light red, contain multiple foci of subpleural hemorrhage, and abundant foamy, bloody edematous fluid has been observed in lung sections[22]. As there were forensic cases due to CO poisoning in our study, the autopsy reports of these cases showed swollen and edematous lungs, brown in COVID-19 cases and smokers, and light red in other cases. In addition, bloody and foamy edema fluid was present, especially in COVID-19 patients.

COVID-19 may present with clinical manifestations ranging from mild upper respiratory tract symptoms to interstitial pneumonia[23]. They are specifically targeting COVID-19 and type II alveolar cells[24]. In those who survive COVID-19, gas exchange abnormalities may develop due to abnormal alveolar injury healing, loss of pulmonary vascular bed, or both[25]. It is known that loss of pulmonary vasoregulation causes hypoxia. In patients with COVID-19 pneumonia, especially in the early stages, hypoxemia is more severe than would be predicted based on anatomical shunts[26,27]. This is because the primary change in pulmonary perfusion results in deep ventilation/perfusion inequalities[28]. Gattinoni *et al*[28] demonstrated by computed tomography (CT) that early-phase lungs (Type L/Type I) are characterized by low elasticity, collectibility, and ventilation/perfusion ratio. COVID-19 has been documented to exhibit microvascular thrombosis. Pulmonary microvascular thrombosis, characterized by subsegmental vascular enlargement and elevated D-Dimer levels near areas of opacities on thorax CT, has been consistently reported and has been linked to increased mortality[29,30].

Although the recovery period for COVID-19 varies according to the severity of the disease, recovery can take a few weeks to a few months. It is not yet known how much damage will occur in which organ after the disease. In the early period of recovery, more than half of COVID-19 patients have impaired diffusion capacity, decreased respiratory muscle strength, and abnormalities in lung imaging. Severe cases are associated with greater reductions in total lung capacity, CO diffusion capacity, and the six-minute walk test[31]. Four months after COVID-19, severe cases had a lower PaO_2 than mild/moderate cases. During acute COVID-19, various measures of pulmonary function at follow-up were negatively correlated with mechanical ventilation duration, CO diffusion capacity, and total lung capacity in subjects requiring mechanical ventilation[32]. CO diffusing capacity dysfunction has been demonstrated in COVID patients at discharge and one month later. According to their study, abnormalities in CO diffusing capacity were noted in 47.2% and 52.6% of patients, respectively. Significant differences in impaired diffusion capacity have been reported between different severe COVID-19 groups[13,33].

Both our study and studies in the literature showed that while COVID-19 itself causes a decrease in lung diffusion capacity, CO poisoning also contributes to this. Thus, both conditions cause a significant decrease in the lung diffusion capacity of CO and an increase in the mortality rate. Recent studies, similar to our study, have shown that approximately half of discharged patients have residual abnormalities on chest CT scans[34]. These studies have shown that approximately three-quarters of COVID-19 patients develop pulmonary dysfunction during early convalescence, the most common being impaired diffusion capacity and decreased forced expiratory volume/forced vital capacity ratio. More than half of COVID-19 patients appear to have CO lung diffusion capacity abnormalities and impaired intra-alveolar diffusion pathways. Impaired CO lung diffusion capacity is the most common abnormality, even in severe acute respiratory syndrome survivors, ranging from 15.5% to 43.6%[35-39]. Mild to moderate cases are more likely to have CO

Table 3 Univariate and multivariate analysis of variables in patient groups

Patient groups	Univariate				Multivariate			
	R square	β	*t*	*P* value	R square	β	*t*	*P* value
COHb (%)	0.452	0.672	19.837	**< 0.001**	0.589	0.166	2.287	**0.023**
Troponin (ng/mL)	0.485	0.696	21.197	**< 0.001**		0.258	3.457	**0.001**
Lactate (mmol/L)	0.468	0.684	20.501	**< 0.001**		0.241	2.462	**0.014**
$PaCO_2$ (mmHg)	0.364	0.604	16.535	**< 0.001**		0.338	6.040	**< 0.001**
Bicarbonate (mmol/L)	0.309	-0.556	-14.659	**< 0.001**		0.259	2.306	**0.022**
Calcium (mmol/L)	0.028	-0.168	-3.723	**< 0.001**		0.072	2.141	**0.033**
Glucose (mg/dL)	0.243	0.494	12.420	**< 0.001**		0.166	3.532	**< 0.001**
Age (year)	0.187	0.433	10.483	**< 0.001**				
pH	0.386	-0.621	-17.319	**< 0.001**				
PaO_2 (mmHg)	0.178	-0.422	-10.164	**< 0.001**				
Potassium (mmol/L)	0.233	0.482	12.031	**< 0.001**				
Sodium (mmol/L)	0.009	-0.095	-2.083	**0.038**				
Base excess (mmol/L)	0.317	0.563	14.480	**< 0.001**				

Bold values indicate significance at $P < 0.05$. COHb: Carboxyhemoglobin; $PaCO_2$: Partial pressure of carbon dioxide; pH: Potential of hydrogen; PaO_2: Partial arterial oxygen pressure.

Table 4 Receiver operating characteristic curve of mortality

	Sensitivity (%)	Specificity (%)	AUC	95%CI	*P* value
COHb (%)	98.4	97.1	0.989	0.980-0.999	< 0.001
Troponin (ng/mL)	98.6	97.8	0.996	0.993-1.000	< 0.001
Lactate (mmol/L)	99.5	98.9	1.000	1.000-1.000	< 0.001

COHb: Carboxyhemoglobin; AUC: Area under the curve; CI: Confidence interval.

lung diffusion capacity abnormalities compared with severe patients[40].

Recently, it was reported that concentrations of particles smaller than particulate matter 2.5 (PM2.5), CO and ozone produced by wildfires are associated with increases in COVID-19 cases and deaths in various parts of California[41,42]. Environmental pollution of PM2.5, CO and ozone can act as a carrier of infection, impair immunity, make humans more susceptible to pathogens, and is an aggravating pathogenic factor for disease[43]. It has been reported that there is a relationship between the severity of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection and air pollution. Among the mechanisms by which air pollution may facilitate SARS-CoV-2 infection is a possible link between upregulation of the angiotensin-converting enzyme receptor by air pollution and host susceptibility to more severe COVID-19. In addition, CO is a highly toxic gas that can damage the lungs[44]. These mechanisms, consistent with our study, support the hypothesis that CO, one of the most important environmental pollutant particles, causes an increase in SARS-CoV-2 cases and deaths.

According to this information, we can say that COVID-19 causes a decrease in diffusion capacity and lung functions with subsequent deterioration in alveolar structure. Although CO diffusion capacity was not measured in our study, pH, $PaCO_2$, and PaO_2 levels in arterial blood gas were evaluated. While blood gas parameters were close to normal in the pre-pandemic group, respiratory acidosis, hypercarbia, and low PaO_2 levels were found in the pandemic group.

In this study, as expected, the COHb and pH values were similar in those who did not have a history of COVID-19 in the pre-pandemic period and during the pandemic period. However, both increased levels of COHb and more acidic pH values were observed in proportion to the severity of the disease in the group of patients who were positive and had COVID-19. Both ICU admission and mortality were observed to be higher in CO poisoning during the pandemic period. Both COHb levels and survival status were strongly correlated with patients' status during or before the pandemic. Considering the pathophysiology of COVID and CO poisoning in the light of the above information, we think that the diffusion mechanism of COHb is impaired and its levels increase more easily due to the influence of the pulmonary airways and alveolar structure in patients with a history of COVID, which causes the patients' clinical worsening.

Table 5 Spearman's rho correlation analysis of variables in patient and survival groups

Spearman's rho	Patients		Survival	
	R	*P* value	R	*P* value
Age (year)	0.441	< 0.001	0.480	< 0.001
Exposure time (hour)	0.148	< 0.001	0.290	< 0.001
COHb (%)	0.609	< 0.001	0.873	< 0.001
Troponin (ng/mL)	0.608	< 0.001	0.807	< 0.001
Lactate (mmol/L)	0.643	< 0.001	0.880	< 0.001
pH	-0.595	< 0.001	-0.857	< 0.001
$PaCO_2$ (mmHg)	0.571	< 0.001	0.801	< 0.001
PaO_2 (mmHg)	-0.495	< 0.001	-0.747	< 0.001
Glucose (mg/dL)	0.467	< 0.001	0.432	< 0.001
Bicarbonate (mmol/L)	-0.536	< 0.001	-0.834	< 0.001
Base excess (mmol/L)	0.541	< 0.001	0.839	< 0.001

COHb: Carboxyhemoglobin; pH: Potential of hydrogen; $PaCO_2$: Partial pressure of carbon dioxide; PaO_2: Partial arterial oxygen pressure.

It should be mentioned that pH, $PaCO_2$, PaO_2, blood sugar, HCO_3, base deficit, lactate, COHb and troponin I values in arterial blood gas parameters of patients show that mortality will be high if CO poisoning is present with COVID-19. It was observed that the mortality rate was 6.4 times higher than the normal population if the high ICU rate was accompanied by high blood sugar, COHb, troponin I and lactate levels in patients aged 60 years and above. In the uni-multivariate analysis, these parameters can have a predictive value in the presence of both CO and COVID-19. In addition, it was determined that these parameters were also correlated with mortality and both the sensitivity and specificity values were above 95%.

This study has some limitations. The most important of these was that this was a retrospective single-center study. In addition, not knowing exactly how much CO the patients were exposed to and for how long was due to difficulties in accessing file data, and arterial blood gas results can be counted among other limitations.

CONCLUSION

CO poisoning has been associated with more severe clinical and biochemical abnormalities, as well as a higher rate of mortality, in individuals with a history of COVID-19. We anticipate that this will have important consequences for the future diagnosis and treatment of COVID-19, as CO levels may be abnormal in comparison to healthy persons and can also be higher in mechanically ventilated patients. Furthermore, we believe that relying on pulse oximeters to determine oxygen saturation is unreasonable, and that doctors should produce more precise data using technologies that can discern levels in the lungs, arteries, and the mean of all tissues. In terms of practicality, this is the simplest arterial blood gas measurement. CO alterations may occur as a result of lung structural disorder during external poisonings, as well as COVID pathology, which can elevate CO levels. Further investigations are required to clarify these issues.

ARTICLE HIGHLIGHTS

Research background

There is a need for new techniques to assess risk in patients with both coronavirus disease 2019 (COVID-19) and carbon monoxide (CO) poisoning, and techniques to aid rapid diagnosis.

Research motivation

The impact of emergency room patients with COVID-19 and CO poisoning on clinical status, morbidity and morbidity is worth investigating.

Research objectives

We aim to determine whether patients with COVID-19 and CO poisoning, as the primary outcome, are definite risk factors for short-term emergency hospitalization and whether there is long-term morbidity and mortality during hospitalization as a secondary outcome.

Research methods

This single-center retrospective study was conducted between January 2018 and December 2021, and included 479 CO poisoning patients. The patients were divided according to the pandemic period and the pre-pandemic period. In addition, the pandemic period was classified according to the presence of COVID-19 and its clinical severity. Patients' demographic, clinical, arterial blood gas, COVID-19 polymerase chain reaction, and other laboratory data were extracted from the hospital information system.

Research results

The mean age of the 479 patients was 54.93 ± 11.51 years, and 187 (39%) were female. 226 (47%) patients were included in the pandemic group and 143 (30%) of them had a history of COVID-19. The mean potential of hydrogen (pH) in arterial blood gas of all patients was 7.28 ± 0.15, was 7.35 ± 0.10 in the pre-pandemic group, and was 7.05 ± 0.16 in the severe group during the pandemic period ($P < 0.001$). Carboxyhemoglobin (COHb) was 23.98% ± 4.19% in the outpatients and 45.26% ± 3.19% in the mortality group ($P < 0.001$). Partial arterial oxygen pressure (PaO_2) was 89.63 ± 7.62 mmHg in the pre-pandemic group, and 79.50 ± 7.18 mmHg in the severe group during the pandemic ($P < 0.001$). While 35 (7%) of all cases died, 30 (85.7%) of those that died were in the pandemic group ($P < 0.001$). In the univariate linear analysis, the relationship between COHb, troponin, lactate, partial arterial pressure of carbon dioxide, bicarbonate, calcium, glucose, age, pH, PaO_2, potassium, sodium, and base excess levels was statistically significant with the pre-pandemic and pandemic groups. In the receiver operating characteristic curve analysis, changes in COHb, lactate, and troponin due to CO poisoning were determined. Based on this analysis, the optimum cut-off values (sensitivity and specificity), the area under the curve, and the 95% confidence interval for COHb, lactate, and troponin were found to be above 45% in predicting the evolution of the pre-pandemic and pandemic groups ($P < 0.001$).

Research conclusions

In cases with a history of COVID-19, CO poisoning was observed with more severe clinical and laboratory findings and more frequent mortality. We believe this will have critical implications for the diagnosis and treatment of COVID-19 in the future, as CO levels may be abnormal compared to healthy subjects and can be higher in mechanically ventilated patients.

Research perspectives

CO poisoning in the pre-pandemic period appears to be milder than in the pandemic period. However, it was determined that mortality due to CO poisoning during the pandemic period was much higher in COVID-19 patients with a moderate and severe clinical course.

FOOTNOTES

Author contributions: Coskun A and Demirci B contributed to study design, concept, writing the manuscript, and revising the final form; Coskun A and Turkdogan KA contributed to data collection and manuscript revision; All authors contributed to writing and discussion management; All authors contributed to data management and manuscript revision, data collection, interpretation of data, and revising the manuscript; Coskun A contributed to data collection and revision; Turkdogan KA contributed to data collection; Demirci B contributed to critical revision; Turkdogan KA contributed to statistical analysis; Coskun A suggested the idea, as a chair of the department provided general support and substantial contribution to concept and design, and acquisition of data; All authors read and approved the final manuscript.

Institutional review board statement: All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the last Declaration of Helsinki (2013), and the protocol was approved by the Ethics Committee of Project identification (Decision No: 136).

Conflict-of-interest statement: The authors report there are no competing interests to declare.

Data sharing statement: Informed Consent Form belonging to the research titled "Clinical Relation of Carboxyhemoglobin Levels in Carbon Monoxide Poisonings with COVID-19", which I conducted, was uploaded to the approved system on April 22, 2022.

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Country/Territory of origin: Turkey

ORCID number: Abuzer Coskun 0000-0003-4824-7021; Burak Demirci 0000-0001-6658-7260; Kenan Ahmet Turkdogan 0000-0003-4850-5094.

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ORIGINAL ARTICLE

Observational Study

External validation of the Moroccan Arabic version of the European Organization for Research and Treatment of Cancer colorectal (CR29) module: Monocentric study

Houda Bachri, Hajar Essangri, Nezha El Bahaoui, Amine Benkabbou, Raouf Mohsine, Anass Mohammed Majbar, Amine Souadka

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Houda Bachri, Hajar Essangri, Nezha El Bahaoui, Amine Benkabbou, Raouf Mohsine, Anass Mohammed Majbar, Amine Souadka, Surgical Oncology Department, National Institute of Oncology, Mohammed Vth University in Rabat, Rabat 10100, Morocco

Hajar Essangri, Gynecology oncology Department, Royal London Hospital, Barts NHS Health, London SE18 6EY, United Kingdom

Corresponding author: Amine Souadka, MD, PhD, Professor, Surgical Oncology Department, National Institute of Oncology, Mohammed Vth University in Rabat, Hay riad Souissi, Rabat 10100, Morocco. a.souadka@um5r.ac.ma

Abstract

BACKGROUND

Quality of life (QoL) outcomes are a focal endpoint of cancer treatment strategies.

AIM

To externally validate the Moroccan Arabic version of the European Organization for Research and Treatment of Cancer (EORTC) QoL Questionnaire (QLQ) for colorectal cancer (CRC) patients (CR29).

METHODS

Both Moroccan Arabic modules of QLQ-CR29 and QLQ-C30 were administered to Moroccan CRC. Psychometric properties were retested by measuring Cronbach's alpha coefficient for reliability and Intraclass correlation coefficient (ICC) to examine test-retest reproducibility. The multitrait-scaling analysis was performed to demonstrate the validity of the instrument and known-groups comparison was used to test the score's ability to discriminate between different groups of patients.

RESULTS

In total, 221 patients were included in our study and 34 patients completed the questionnaire twice. The Urinary Frequency scale and Stool Frequency scale had good internal consistency with alpha Cronbach coefficients of 0.79 and 0.83 respectively, while the same coefficients were moderately lower for the Blood and Mucus in Stool scale (0.61) and the Body Image scale (0.67). The ICCs ranged from

0.88 to 1 indicating good to excellent reproducibility. In multitrait scaling analyses, the criterion for item convergent and divergent validity was satisfactory. The known-group comparison showed statistically significant differences between patients according to age, gender, stoma status, tumor location, and radiotherapy.

CONCLUSION

The Moroccan Arabic version of the EORTC QLQ-CR29 is a valid and reliable tool that can be used safely for research and clinical purposes in Moroccan CRC patients.

Key Words: Rectal neoplasm; Colorectal cancer; Health-related quality of life; Patient reported outcome measures; European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-CR29; European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-C30

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Core Tip: Patient related outcomes such as quality of life (QoL) are a focal endpoint of cancer treatments strategies. Many QoL Questionnaire (QLQ) are not trully validated. We aim to externally validate the Moroccan Arabic version of the European Organization for Research and Treatment of Cancer QLQ CR29 on larger and more heterogenous population in order to affirm its validity and reliability in arabic colorectal cancer patients.

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INTRODUCTION

Colorectal cancer (CRC) is a global challenge[1]. However, even with an increasing incidence, the implementation of screening programmes and the large array of advanced treatment modalities has significantly reduced mortality[2,3]. Nonetheless, CRC survivors suffer impaired physical and bowel functions, as well as psychological symptoms such as anxiety, sleep disruption, and depression[4]. All together, these symptoms negatively reflect on the quality of life (QoL)[5] and makes looking beyond oncological outcomes of great importance.

Health-related QoL (HRQL) is an abstract and multidimensional concept[6] which can be assessed by the European Organization for Research and Treatment of Cancer (EORTC) questionnaires. Core measurement tools examine issues common to different cancer sites and can be used as a stand-alone questionnaire or in combination with disease specific modules[7]. The EORTC QoL Questionnaire (QLQ) CR29 questionnaire specific to CRC and its psychometric properties have been tested in several languages and contexts[8-16].

Recently, The QLQ-CR29 has just been only translated for Moroccan Arabic dialect[17]. However this adaptation was performed on a very limited sample size of 120 patients under the usual requests of the EORTC organization. The aim of this study is to externally validate this version and assess its psychometric properties on larger Moroccan CRC patients.

MATERIALS AND METHODS

Description of the instruments

We followed the STROBE directive guidelines write the manuscripts[18]. The participants completed a general information section including sociodemographic and clinical data, alongside both the Moroccan Arabic module of EORTC QLQ-CR29[18], and the validated Moroccan Arabic version of the QLQ-C30 (version 3.0)[19].

The EORTC QLQ-C30

The EORTC QLQ-C30 includes five functional subscales (*i.e.*, physical functioning, role functioning, emotional functioning, cognitive functioning, and social functioning), three symptom subscales (*i.e.*, fatigue, nausea and vomiting, and pain), a global QoL subscale, and six single symptom items (*i.e.*, dyspnea, insomnia, appetite loss, constipation, diarrhea, and financial difficulties). The scoring of items is on a 1 to 7 and 1 to 4 Likert scales for the global health status/QoL and the other scales. High scores represent better functioning and worse symptoms[20,21].

The EORTC QLQ-CR29

The morocan arabic module of EORTC QLQ-CR29[17], is a colon and rectum site-specific QoL module with 29 items

consisting of 4 multi-item scales (body image, urinary frequency, blood and mucus in stool, and stool frequency) and 17 functional/symptomatic single-items (sexual interest, urinary incontinence, dysuria, abdominal pain, buttock pain, bloating, dry mouth, hair loss, taste, flatulence, fecal incontinence, sore skin, embarrassment, stoma care problem, impotence or dyspareunia). Among these items, only body image, anxiety, weight, and sexual interest are functional scales.

The eighteenth item (Q18) is an indicator of colostomy/ileostomy construction, while the following items are separately arranged for patients with a stoma (Q19-Q25) and without (Q19-Q25) according to symptoms of stool frequency, flatulence, fecal incontinence, sore skin and embarrassment while item 25 is specific for stoma care. Sexual interest, impotence and dyspareunia items are categorized according to gender with the corresponding questions being Q26-Q27 and Q28-Q29 for male and female respondents respectively. All questionnaire items ask about the past week except the ones on sexuality, which request the patients to evaluate the past four weeks. As regards the scoring, the multi-item scales and single items are scored using a 1 to 4 point Likert scale ("not at all", "a little", "quite a bit", "very much") with the highest score representing the best functional status or the worst symptom[22].

Study population and data collection

Patients were prospectively recruited from the national oncology institute during the period between November 2019 and January 2020[23,24]. Patients aged over 18 years old, with pathologically confirmed colon and/or rectum cancer and who underwent surgery at least 6 mo prior to the enrollment in the study were included. Patients were excluded if they were unable to understand the questionnaire, had cognitive and/or medical complications that hindered the interview completion and those who submitted an incomplete questionnaire. Participants were either approached during follow up visits or contacted *via* telephone. Patient's characteristics were reported according to age, gender, stoma status, cancer location (colon *vs* rectum), neoadjuvant radiochemotherapy and adjuvant chemotherapy.

As the sample size determination for psychometric validation studies lacks clear recommendations[25], we determined the required sample by allocating a number of observations 5 to 10 times greater than the variables[26]. Accordingly, the sample needed size ranged between 150 and 300 participants in order to externally validate this version.

Statistical analysis

The scores for the QLQ-CR29 and the QLQ-C30 questionnaires were linearly converted into 0 to 100 point scores according to the standard EORTC guidelines[20]. Descriptive statistics were generated through mean, median, standard deviation, and floor and ceiling effects, while age was categorized in 3 groups: < 40 years ; 41- 65 years and > 65 years.

In order to proceed to the external validation of the Moroccan Arabic module of de QLQ-CR29 we followed the identical steps of a first validation in a totally different population. There are two different levels of reliability, namely internal consistency and reproducibility. Internal consistency reliability was determined using Cronbach's alpha coefficient with a score greater than 0.7 considered acceptable, above 0.8 was good and higher than 0.9 was considered excellent.

A random subgroup of patients was selected to retake the QLQ CR-29 questionnaire after 7 to 14 d from the first interview in order to examine the test-retest reliability. The results of the two measurements were assessed using the Intraclass correlation coefficient (ICC) and an ICC score of 0.7 or higher was considered acceptable.

We tested the construct validity of the EORTC QLQ-CR29 using multitrait scaling analysis[27]. Convergent validity was examined by correlating each item with its own scale with an item-scale correlation of ≥ 0.40 equivalent to high correlation. Divergent validity on the other hand was tested by demonstrating that the item correlated higher with its own scale than with the others.

Concurrent validity was examined by comparing the scores of the QLQ-CR29 and the QLQ-C30 using Pearson's correlation.

Clinical validity was assessed using known group comparison through the Mann Whitney U test to examine the QLQ-CR29' ability to differentiate clinically distinct patients. Subgroups were categorized according to: Age (< 65 years *vs* ≥ 65 years), gender (male *vs* female), stoma status (permanent *vs* no stoma), tumor site (colon *vs* rectal) and neoadjuvant radiotherapy (no *vs* yes). All statistical analyses were performed using SPSS 26.0 (SPSS Inc., Chicago,IL, United States). Statistically significant results were defined with a $P < 0.05$.

RESULTS

Patients characteristics

The sociodemographic and clinical characteristics of the patients enrolled in the study are detailed in Table 1. In total, 221 of 250 (88,4%) patients completed the questionnaire among which 123 were males and 98 were females. The mean age of our patients was 55.6 ±12.7years. Seventy-eight (35.9%) participants had colonic cancer and 138 (64.1%) had rectal cancer of wich 89 (64%) received neoadjuvant chemoradiotherapy, while 50 patients had a stoma (22,6%). Missing items were only associated with sexual problems with a miss rate of 9% for males and 23% for females.

Table 2 summarizes the distribution of the EORTC QLQ CR-29 and QLQ-C30 scores. The mean score for the different dimensions of the QLQ CR-29 ranged from 16.44 to 75.56 with the items "Hair loss" and "Weight" scoring the lowest and highest respectively. The percentage of respondents at floor was high (> 50%) in 12 areas while the percentage of respondents at ceiling was high (> 50%) in 1 item. The range of scores was broad in 21 dimensions except for the bag change it ranged from 0 to 83.

Table 1 Patients clinical and demographic characteristics

Variables	Description
Age, mean ± SD	55.65 ± 12.87
Sex	
Female	98 (44.5%)
Male	123 (55.5%)
Tumor location	
Colon	78 (35%)
Rectum	139 (62%)
Neoadjuvant chemoradiotherapy	
No	107 (54,6%)
Yes	89 (45,5%)
Definitive stoma	
No	50 (22.6%)
Yes	171 (77.4%)
Adjuvant chemotherapy[a]	
Yes	91 (70%)
No	39 (30%)

[a]Missing data in this variable.

Reliability

The internal consistency of the EORTC QLQ-CR29 reached the 0.7 criterion showing good consistency for the urinary frequency scale (0.79) and stool frequency scale (0.83), while for the blood and mucus (0.615) and the body image (0.672) scales the alpha Cronbach coefficient was slightly below the criterion (0.7). The Cronbach's alpha coefficient was higher for patients without stoma compared to those with stoma, except for the body image scale (0.64 with *vs* 0.69 without) which indicates higher reliability for patients without a stoma. More details are shown in Table 3.

Thirty four patients took the Arabic version of the QLQ-CR29 and for each item, the ICCs ranged from 0.889 to 0.999 indicating good to excellent reproducibility.

Construct validity

All items exceeded the 0.40 criterion for item-scale convergent validity. Similarly, items correlated better with their own scales than with others which shows good divergent validity. Details of the multitrait scaling analysis are shown in Table 3.

Concurrent validity

Correlations between the scales of the QLQ-CR29 and QLQ-C30 were low ($r < 0.40$). However, some areas with more related content showed higher correlations ($r > 0.40$), namely body image and social functioning. The abdominal pain scale also had a good correlation with the QLQ-C30 pain scale and stoma care problems were correlated to the global QoL scale. In addition, most functional scales of the QLQ-CR29 were positively correlated with functional scales of the QLQ-C30 and negatively correlated with symptom scales of the QLQ-C30, while most symptom scales of the QLQ-CR29 were positively correlated with symptom scales of the QLQ-C30 and negatively correlated with functional scales of the QLQ-C30 as detailed in Table 4.

Clinical validity

The EORTC QLQ-CR29 allowed the distinction between patients based on differences between known groups (Tables 5 and 6).

Differences in the scores of patients with stoma were noted as they presented significantly more anxiety and body image issues. Males with stoma reported higher symptom scores for the "impotence" scale.

The participants with rectal cancer had worse QoL than those with colon cancer and male patients with rectal cancer had significantly higher symptom scores for flatulence, fecal incontinence, sore skin around the anus, stool frequency, defecation problems, and sexual dysfunction.

In addition, patients who received neoadjuvant radiotherapy had significantly higher symptom scores and more problems related to blood and mucus, buttock pain, bloating, stoma care problems, flatulence, fecal incontinence, sore skin, stool frequency, embarrassment and defecation problems.

Table 2 Quality of life scores according to European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-C30 and Quality of Life Questionnaire-CR29 structure and reliability

Scaling/single-item name	*n*	Item No.	Mean	SD	Floor	Ceiling	Range	ICC
EORTC QLQ-CR29								
All patients	221							
Urinary frequency		31.32	39.89	33.46	26.2	10.9	0-100	0.961
Blood & mucus in stool		38.39	24.73	29.02	43.4	4.1	0-100	0.969
(F) Body image		45-47	77.82	24.83	1.8	38	0-100	0.950
Defecation/stoma problems		49–54	-	-	-	-	-	
Urinary incontinence		33	20.96	32.22	64.7	7.2	0-100	0.982
Dysuria		34	20.66	31.30	63.3	6.8	0-100	0.950
Abdominal pain		35	30.61	34.12	48	8.6	0-100	0.922
Buttock pain		36	27.14	34.62	55.7	9	0-100	0.921
Bloated feeling		37	28.80	33.77	50.2	8.6	0-100	0.945
Dry mouth		40	24.58	34.14	60.2	8.6	0-100	0.979
Hair loss		41	16.44	29.57	71.5	5.9	0-100	0.968
Trouble with taste		42	20.51	32.89	67.0	8.1	0-100	0.975
(F) Anxiety		43	64.67	37.60	16.7	43.4	0-100	0.951
(F) Weight		44	75.56	32.66	8.1	56.6	0-100	0.960
Patients with stoma	50							
Flatulence		49s	41.49	33.00	28.6	10.2	0-100	0.908
Leakage		50 s	42.17	36.49	32.7	16.3	0-100	0.889
Sore skin around stoma		51s	42.85	38.49	34.5	20.4	0-100	0.965
Bags change		52.53 s	18.36	22.62	49	2	0-83	0.969
Embarrassed		54s	45.56	43.09	41.8	29.1	0-100	0.956
Stoma care pb		55s	40.08	41.47	46.8	21.5	0-100	0.912
Stoma pb		49-54s	37.41	20.18	4.1	4.1	0-100	0.999
Patients without stoma	172							
Flatulence		49	30.62	37.01	52.9	12.8	0-100	0.980
Faecal incontinence		50	26.16	37.38	61.6	14.0	0-100	0.970
Sore skin around anus		51	20.34	31.72	64.5	7.6	0-100	0.979
Stool frequency		52.53	29.65	32.33	38.4	7.6	0-100	0.977
Embarrassment		54	31.20	38.51	54.7	15.1	0-100	0.975
Defecation pb		49-54	28.79	25.84	16.1	0.7	0-100	0.969
Male	123							
Sexual functioning		56	42.85	37.81	33	20.5	0-100	0.928
Impotence		57	38.18	38.79	40.9	20	0-100	0.966
Female	98							
Sexual functioning		58	67.06	36.76	11.9	48.8	0-100	0.933
Dyspareunia		59	26.58	35.75	58.3	10.7	0-100	0.985
C30	221							
Physical function		1 - 5	73.64	23.85	0.9	23.9	0-100	-
Role function		6.7	62.92	37.00	13.3	39.0	0-100	-

Emotional function	21-24	67.24	30.77	4.1	30.7	0-100	-
Cognitive function	20. 25	83.94	23.45	0.5	58.3	0-100	-
Social function	26 . 27	79.58	28.93	3.7	57.3	0-100	-
Fatigue	10.12.18	30.98	29.17	27.1	2.8	0-100	-
Nausea and vomiting	14.15	7.79	17.16	78.0	0.5	0-83	-
Pain	9.19	24.31	29.72	46.8	3.7	0-100	-
Dyspnoea	8	21.10	30.59	62.4	4.6	0-100	-
Insomnia	11	27.67	35.25	56.4	9.2	0-100	-
Appetite loss	13	20.48	30.99	63.3	6.4	0-100	-
Constipation	16	27.52	33.97	53.8	1.4	0-100	-
Diarrhea	17	27.67	34.67	53.2	10.6	0-100	-
Financial difficulties	28	51.22	40.61	30.7	30.7	0-100	-

EORTC: European Organization for Research and Treatment of Cancer; QLQ: Quality of Life Questionnaire.

Table 3 Convergent and discriminant validity of the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-CR29

QLQ-CR29 scales	Total sample (*n* = 221)			Patients without stoma (*n* = 50)			Patients with stoma (*n* = 171)		
	Convergent	Discriminant	α	Convergent	Discriminant	α	Convergent	Discriminant	α
Urinary frequency	0.905-0.907	-0.00-0.25	0.795	0.83-0.84	-0.00-0.21	0.66	0.91-0.92	-0.12-0.25	0.82
Blood or mucus in stool	0.74-0.89	-0.27-0.35	0.615	0.62-0.96	-0.44-0.35	0.581	0.79-0.87	-0.20-0.30	0.65
Body image	-0.66-0.75	-0.00-0.36	0.672	-0.51-0.89	-0.20-0.39	0.690	-0.63-0.72	-0.07-0.19	0.64
Stool frequency	0.83-0.96	-0.30-0.39	0.835[1]	0.83-0.96	-0.31-0.39	0.804	-0.85-0.91	-0.14-0.34	0.87

[1]Mean of cronbach's alpha coefficient for patients without and with stoma.
Multitrait scaling analysis' summary of the results; ranges for convergent and discriminant validity of each multiitem scale and their internal consistency using cronbach's alpha. QLQ: Quality of Life Questionnaire.

Furthermore, the QLQ-CR29 showed differences between age groups with younger patients found to suffer more from defecation problems, stool frequency and embarrassment.

DISCUSSION

HRQL in CRC is an important component in both day to day practice and clinical research, therefore the proper assessment of patients' HRQOL is crucial[28]. This study showed that the Arabic version of the EORTC QLQ-CR29 questionnaire has good internal consistency, test-retest reliability and validity and is therefore valid and reliable to assess the QoL of Moroccan CRC patients.

The internal consistency of the Arabic EORTC QLQ CR-29 demonstrated satisfactory results for the urinary frequency scale and stool frequency scale, with higher reliability scores for patients without a stoma which is similar to the Chinese validation[14]. As regards the blood and mucus and the body image scales, the alpha Cronbach coefficients were acceptable which was the case in other similar studies[12,29]. On the other hand, as suggested by Arraras *et al*[12], some differences may be due to the fact that the EORTC original validation was conducted on an international sample with high variance, while the Spanish validation concerned a more homogenous sample which may impact the alpha Cronbach coefficient.

The ICCs of our study were all greater than 0.8, thus indicating good to excellent reproducibility for both single item and multi-item scales. The Reliability coefficients were higher in our study than those reported by the Dutch validation [10] and mostly similar to those in the original psychometric validation study[8]. As such, the Moroccan Arabic translation of the QLQ CR-29 is a stable instrument.

The multitrait analysis confirmed the structure of all scales, which proves that the Moroccan Arabic translation of the QLQ-CR29 has a valid construct.

Table 4 Correlation between the Quality of Life Questionnaire-CR29 and the Quality of Life Questionnaire-C30

	EORTC QLQ C30														
CR-29	Functional scales						Symptom scales								
Scales/Single items	QoL	PF	RF	EF	CF	SF	FA	NV	PA	DY	SL	AP	CO	DI	FI
Functional scales															
Body image	0.294[2]	0.279[2]	0.370[2]	0.214[2]	0.244[2]	0.403[2]	-0.298[2]	-0.256[2]	-0.161[1]	-0.250[2]	-0.151[1]	-0.278[2]	-0.221[2]	-0.003	-0.079
Anxiety	0.297[2]	0.314[2]	0.264[2]	0.315[2]	0.273[2]	0.285[2]	-0.294[2]	-0.160[1]	-0.210[2]	-0.169[1]	-0.138[1]	-0.117	-0.029	-0.008	-0.167[1]
Sexual function: Male	-0.121	-0.133	-0.058	-0.011	-0.034	-0.111	0.009	-0.010	0.084	0.089	0.124	0.109	0.049	0.049	0.018
Sexual function: Female	-0.299[2]	-0.192	-0.115	-0.079	-0.256[1]	0.040	0.082	-0.033	0.221[1]	-0.001	0.230[1]	0.040	0.017	0.112	0.033
Symptom scales															
Urinary frequency	-0.137[1]	-0.247[2]	-0.201[2]	-0.237[2]	-0.089	0.029	0.244[2]	0.070	0.218[2]	0.230[2]	0.176[2]	0.165[1]	0.042	0.221[2]	0.122
Blood and mucus in stool	-0.241[2]	-0.283[2]	-0.269[2]	-0.190[2]	-0.110	-0.123	0.359[2]	0.215[2]	0.349[2]	0.190[2]	0.277[2]	0.268[2]	0.152[1]	0.302[2]	0.256[2]
Urinary incontinence	-0.009	-0.128	-0.060	-0.237[2]	-0.152[1]	-0.050	0.102	0.014	0.108	0.150[1]	0.195[2]	0.030	0.135[1]	0.032	0.044
Dysuria	-0.017	-0.103	-0.161[1]	-0.069	-0.065	-0.047	0.153[1]	0.012	0.172[1]	0.058	0.133[1]	0.086	0.091	0.100	0.025
Abdominal pain	-0.138[1]	-0.161[1]	-0.099	-0.125	-0.099	-0.055	0.232[2]	0.143[1]	0.443[2]	0.140[1]	0.254[2]	0.122	0.168[1]	0.107	-0.039
Buttock pain	-0.212[2]	-0.265[2]	-0.270[2]	-0.103	-0.098	-0.074	0.363[2]	0.149[1]	0.469[2]	0.194[2]	0.253[2]	0.190[2]	0.025	0.149[1]	0.280[2]
Bloated feeling	-0.206[2]	-0.213[2]	-0.138[1]	-0.213[2]	-0.145[1]	-0.084	0.292[2]	0.171[1]	0.377[2]	0.256[2]	0.380[2]	0.058	0.253[2]	0.040	0.073
Dry mouth	-0.309[2]	-0.341[2]	-0.257[2]	-0.266[2]	-0.283[2]	-0.125	0.340[2]	0.390[2]	0.205[2]	0.202[2]	0.141[1]	0.329[2]	0.211[2]	0.145[1]	0.113
Hair loss	-0.036	-0.195[2]	-0.133[1]	-0.337[2]	-0.242[2]	-0.131	0.183[2]	0.217[2]	0.084	0.080	0.141[1]	0.182[2]	0.200[2]	0.135[1]	0.033
Trouble with taste	-0.099	-0.247[2]	-0.236[2]	-0.134[1]	-0.173[1]	-0.125	0.243[2]	0.343[2]	0.036	0.202[2]	0.010	0.271[2]	0.173[1]	0.101	0.072
Weight	0.176[2]	0.280[2]	0.291[2]	0.157[1]	0.121	0.179[2]	-0.238[2]	-0.128	-0.081	-0.060	-0.143[1]	-0.165[1]	-0.169[1]	-0.083	-0.017
Flatulences	0.124	-0.023	0.139	-0.126	-0.180	-0.260	0.077	0.056	-0.062	0.262	0.042	0.065	0.131	0.147	0.117
Leakage	0.083	0.142	0.046	-0.271	-0.128	-0.146	0.033	0.059	-0.023	0.114	-0.004	0.093	0.029	0.127	0.240
Sore skin around stoma	-0.041	-0.190	0.025	-0.600[2]	-0.402[2]	-0.330[1]	0.295[1]	0.261	0.297[1]	0.247	0.380[2]	0.073	0.172	0.002	0.133
Bags changes	-0.085	-0.169	0.002	-0.404[2]	-0.025	-0.273	0.228	-0.018	0.155	0.218	0.344[1]	0.192	-0.003	0.019	-0.098
Embarrassment	-0.407[2]	-0.312[2]	-0.394[2]	-0.272[1]	-0.150	-0.164	0.476[2]	0.155	0.419[2]	0.325[2]	0.353[2]	0.156	-0.079	0.030	0.361[2]
Stoma care problems	-0.502[2]	-0.391[2]	-0.458[2]	-0.277[1]	-0.182	-0.228[1]	0.529[2]	0.239[1]	0.587[2]	0.328[2]	0.468[2]	0.239[1]	0.093	-0.064	0.343[2]

Stoma problems	-0.077	-0.142	-0.009	-0.580[2]	-0.277	-0.364[1]	0.336[1]	0.108	0.181	0.264	0.301[1]	0.138	0.104	0.179	0.139
Flatulences	-0.160[1]	-0.151[1]	-0.034	-0.149	-0.137	-0.112	0.144	0.032	0.177[1]	0.269[2]	0.173[1]	-0.003	0.008	0.252[2]	0.200[2]
Faecal incontinence	-0.036	-0.081	-0.040	-0.142	-0.122	-0.117	0.153[1]	0.084	0.190[1]	0.141	0.111	-0.017	-0.140	0.403[2]	0.203[2]
Sore skin around anus	-0.081	-0.012	-0.045	-0.057	-0.047	-0.102	0.049	0.113	0.086	0.121	0.027	0.027	-0.006	0.149	0.195[1]
Stool frequency	0.002	-0.019	-0.024	-0.071	0.002	0.024	0.083	-0.044	0.259[2]	0.092	0.123	0.004	-0.035	0.452[2]	0.189[1]
Embarrassment	-0.101	-0.133	-0.135	-0.207[2]	-0.167[1]	-0.208[2]	0.178[1]	0.141	0.149	0.276[2]	0.152[1]	0.062	0.146	0.224[2]	0.203[2]
Defecation	-0.111	-0.116	-0.038	-0.202[1]	-0.159	-0.173[1]	0.190[1]	0.157	0.280[2]	0.316[2]	0.181[1]	0.040	0.106	0.379[2]	0.290[2]
Impotence	0.019	-0.065	-0.149	-0.104	-0.170	-0.218[1]	0.075	0.243[1]	-0.104	0.313[2]	0.065	0.308[2]	0.175	0.008	0.064
Dyspareunia	-0.083	-0.096	-0.118	-0.174	-0.243[1]	-0.345[2]	0.108	0.025	0.260[1]	0.163	0.162	0.156	0.045	0.157	0.236[1]

[1]$P < 0.05$.

[2]$P < 0.01$.

EORTC: European Organization for Research and Treatment of Cancer; QLQ: Quality of Life Questionnaire; QoL: Quality of life; PF: Physical functioning; RF: Role functioning; EF: Emotional functioning CF: Cognitive functioning; SF: Social functioning; FA: Fatigue; NV: Nausea/vomiting; PA: Pain; DY: Dyspnea; SL: Insomnia; AP: Appetite loss; CO: Constipation; DI: Diarrhea; FI: Financial problems.

In the assessment of concurrent validity, correlations between the scales of the QLQ-C30 and the QLQ CR-29 were mostly low (< 0.4) indicating that the two questionnaires measure different concepts. Few areas with related content had higher correlation scores which was expected given the similar concepts of these particular scales. Nonetheless, the results show that the two questionnaires are independent.

In terms of clinical validity, we found less significant differences related to stoma status than the original study[8]. Moreover, patients with colon cancer had a better function and fewer symptoms, including sexual interest in males and stool frequency as opposed to rectal cancer. Interestingly, patients with rectal cancer and a stoma experienced more embarrassment with borderline significance ($P = 0.053$). When comparing age groups, younger patients reported worse symptoms than older patients[30]. Similar results were reported by the Dutch and Spanish Validation studies[10,12]. In addition, the particularities of the Moroccan population may be contributing to elderly patients' display of better resilience, QoL satisfaction, relatively better acceptance and the aforementioned results. Consequently, the QLQ-CR29 was found to discriminate between age groups.

A higher missing data rate was registered for sexual dimensions compared to others as patients were more reticent about answering sex-related questions which makes their interpretation more difficult. Similar observations were made in the Chinese and Iranian studies, which hindered discussions regarding sexual activity and even ostomy[14,16]. Nonetheless, providing explanations to patients when answering the questionnaire was noted to help. In our context, this issue may be explained by the cultural and religious particularities of the Moroccan population where sexual practices are taboo[31]. More studies addressing this problem should be conducted to determine the reliability and validity of the CR-29 in evaluating the sexual aspects of QoL for patients according to cultural contexts.

This study has some limitations, one of which is the limited sample size of patients. However, the minimum sample size was set at one hundred and fifty patients according to EORTC organization and other EORTC QLQ-CR29 validations were performed on a smaller population such as El Alami's research[18]. Self-administration was not possible due to the high level of illiteracy in our context; consequently, patients received the help of one of the investigators who was in

Table 5 Known-group comparisons

	Stoma status			Colon *vs* Rectum			Neoadjuvant radiochemotherapy		
CR-29 scales/single items	Yes (n = 50)	No (n = 171)	P value	Colon (n = 78)	Rectum (n = 139)	P value	Yes (n = 89)	No (n = 107)	P value
Urinary frequency	35.6 (41.1)	41.1 (34.6)	0.412	35.4 (31.2)	42.2 (34.8)	0.215	45.5 (33.9)	35.0 (32.2)	0.031
Blood and mucus in stool	27.6 (28.8)	23.8 (29.1)	0.247	20.2 (26.8)	27.4 (30.2)	0.086	29.9 (30.3)	19.3 (27.1)	0.004
Body image	66.6 (27.5)	81.1 (23.0)	0.000	77.4 (24.8)	77.7 (25.0)	0.938	77.6 (24.1)	78.9 (24.5)	0.625
Urinary incontinence	24.0 (35.0)	20.0 (31.4)	0.451	18.3 (28.7)	21.5 (33.0)	0.788	20.2 (32.0)	21.4 (32.1)	0.592
Dysuria	21.3 (29.1)	20.4 (31.9)	0.469	18.8 (30.6)	22.3 (31.9)	0.120	23.2 (32.3)	18.3 (29.7)	0.299
Abdominal pain	28.0 (32.5)	31.3 (34.6)	0.590	28.6 (34.2)	32.1 (34.3)	0.440	34.4 (34.6)	26.7 (33.4)	0.095
Buttock pain	28.6 (33.6)	26.7 (34.9)	0.563	17.0 (30.26	32.3 (36.1)	0.001	38.5 (36.2)	14.3 (27.5)	0.000
Bloated feeling	30.6 (33.5)	28.2 (33.9)	0.545	27.7 (32.4)	29.0 (34.2)	0.892	35.2 (35.6)	23.6 (31.7)	0.017
Dry mouth	30.0 (36.4)	23.0 (33.4)	0.183	20.5 (29.0)	26.6 (36.5)	0.477	30.7 (38.3)	19.0 (28.9)	0.055
Hair loss	17.3 (28.7)	16.1 (29.1)	0.608	10.6 (24.9)	19.4 (31.5)	0.027	18.7 (30.1)	14.6 (28.6)	0.222
Trouble with taste	27.3 (36.0)	18.5 (31.7)	0.069	13.2 (27.5)	24.4 (35.1)	0.016	20.5 (31.1)	17.7 (31.8)	0.375
Anxiety	52.6 (40.4)	67.8 (36.1)	0.016	63.2 (37.8)	65.4 (37.0)	0.658	65.9 (36.5)	63.5 (38.4)	0.725
Weight	69.3 (38.6)	77.3 (30.6)	0.305	79.4 (30.4)	73.8 (33.2)	0.194	75.6 (30.4)	76.3 (33.9)	0.492
Flatulence				45.8 (31.9)	39.3 (32.7)	0.601	35.0 (34.1)	45.6 (33.7)	0.361
Leakage				45.8 (31.9)	40.4 (38.8)	0.558	42.1 (39.8)	45.6 (31.8)	0.705
Sore skin around stoma				39.5 (32.7)	44.4 (41.3)	0.748	50.8 (43.5)	43.8 (33.4)	0.598
Bags changes				21.8 (27.0)	16.6 (20.4)	0.584	17.5 (19.6)	26.3 (27.3)	0.351
Embarrassment				64.5 (28.46)	40.7 (44.9)	0.056	56.3 (46.3)	36.9 (39.8)	0.055
Stoma care problems				37.5 (38.2)	40.7 (42.5)	0.804	54.0 (42.1)	23.4 (36.7)	0.003
Stoma problems				39.9 (17.1)	36.1 (21.6)	0.499	37.1 (23.4)	41.8 (16.4)	0.387
Flatulences				21.5 (33.6)	34.5 (37.3)	0.017	43.8 (39.1)	19.6 (31.8)	0.000
Faecal incontinence				16.12 (30.6)	31.4 (39.5)	0.009	35.7 (41.0)	17.0 (31.5)	0.001
Sore skin around anus				11.8 (24.2)	25.1 (34.6)	0.012	29.5 (36.1)	13.6 (26.0)	0.002
Stool frequency				19.3 (26.8)	35.8 (34.2)	0.002	37.6 (36.1)	21.5 (26.8)	0.008
Embarrassment				23.1 (35.4)	34.9 (39.1)	0.053	40.4 (39.2)	23.4 (35.7)	0.004
Defecation pb				18.8 (21.5)	36.5 (36.5)	0.000	37.8 (27.4)	19.5 (21.0)	0.000
(F) Sexual function: Male	54.1 (39.1)	39.7 (37.1)	0.162	32.4 (32.4)	48.1 (39.4)	0.047	46.3 (39.4)	36.0 (32.8)	0.211
Impotence	47.2 (39.2)	35.6 (38.5)	0.021	31.5 (34.6)	41.6 (40.6)	0.248	43.9 (39.2)	33.3 (36.8)	0.167
Sexual function: Female	56.8 (38.6)	69.6 (36.1)	0.064	65.4 (33.3)	66.6 (39.6)	0.770	69.4 (35.9)	70.0 (35.2)	0.928
Dyspareunia	33.3 (39.1)	24.8 (34.9)	0.156	20.23 (33.1)	29.4 (37.1)	0.240	26.8 (32.6)	23.3 (35.5)	0.459

charge of reading the questions and different options for the answer. Furthermore, although the use of confirmatory factor analysis may be an option, multitrait scaling analysis is the most frequently used method for the EORTC tools' transcultural validations[21]. Notwithstanding the foregoing, this study clearly validate the Moroccan Arabic validation of the EORTC QLQ-CR29 questionnaire which will allow the correct evaluation of HRQOL of CRC patients.

CONCLUSION

To summarize, the psychometric properties of the Moroccan Arabic version of the EORTC QLQ CR-29 show that it's a reliable and valid instrument to measure the QoL of CRC patients and could be used to complement the EORTC QLQ-C30 in assessing HRQOL. Conducting more transcultural validations and standardizing patient-reported outcome questionnaires, especially in the field of oncology, will allow us to broadly assess cancer therapy outcomes and weigh the benefits against the QoL impact.

Table 6 Known-group comparisons, *P* < 0.0001

	Gender			Age			
CR-29 scales/single Items	Male (*n* = 123)	Female (*n* = 98)	*P* value	≤ 40 (*n* = 26)	41-65 (*n* = 144)	≥ 66 (*n* = 45)	*P* value
Urinary frequency	39.7 (33.1)	40.1 (34.1)	0.862	28.2 (30.4)	42.4 (33.3)	38.1 (34.1)	0.115
Blood and mucus in stool	24.6 (27.8)	24.8 (30.5)	0.794	25.6 (29.5)	26.7 (29.4)	19.2 (27.5)	0.183
Body image	77.4 (25.0)	78.3 (24.7)	0.777	74.3 (23.2)	76.6 (26.1)	80.7 (21.8)	0.481
Urinary incontinence	19.5 (31.9)	22.7 (32.6)	0.385	19.2 (28.5)	19.6 (32.3)	26.6 (33.0)	0.273
Dysuria	23.3 (32.2)	17.3 (29.9)	0.101	23.0 (36.2)	20.8 (31.5)	20.7 (29.5)	0.978
Abdominal pain	27.6 (33.5)	34.3 (34.6)	0.172	32.0 (34.6)	31.9 (35.2)	26.6 (29.8)	0.772
Buttock pain	28.1 (34.9)	25.8 (34.3)	0.478	32.0 (34.6)	27.7 (36.1)	22.2 (30.1)	0.500
Bloated feeling	27.3 (32.8)	30.6 (35.0)	0.495	26.9 (32.6)	31.9 (35.0)	22.9 (30.8)	0.293
Dry mouth	17.3 (28.7)	33.6 (38.1)	0.001	23.0 (33.6)	24.7 (33.8)	25.1 (36.3)	0.967
Hair loss	8.6 (21.7)	26.2 (34.9)	0.000	12.8 (28.4)	19.6 (32.3)	10.3 (19.8)	0.228
Trouble with taste	16.8 (31.4)	25.1 (34.2)	0.040	30.7 (38.7)	20.6 (33.4)	14.8 (27.1)	0.282
Anxiety	69.6 (34.9)	57.8 (39.9)	0.032	65.3 (38.2)	60.1 (38.2)	73.3 (34.5)	0.116
Weight	75.3 (33.3)	75.8 (32.0)	0.920	75.6 (30.6)	74.5 (33.6)	77.0 (31.6)	0.933
Flatulence	43.6 (32.2)	38.3 (34.6)	0.566	38.8 (32.7)	44.7 (33.2)	33.3 (36.5)	0.667
Leakage	47.1 (36.2)	35.0 (36.6)	0.241	61.1 (44.3)	41.9 (34.6)	27.7 (38.9)	0.299
Sore skin around stoma	44.8 (39.1)	40.0 (38.3)	0.650	66.6 (42.1)	42.8 (37.5)	33.3 (36.5)	0.287
Bags changes	20.1 (22.4)	15.8 (23.2)	0.427	22.2 (25.0)	20.9 (23.6)	5.5 (8.6)	0.328
Embarrassment	46.3 (42.4)	44.4 (44.6)	0.680	51.8 (50.3)	51.3 (41.8)	31.4 (40.3)	0.230
Stoma care problems	42.7 (43.1)	36.3 (39.4)	0.481	66.6 (50.0)	40.9 (40.2)	29.6 (37.7)	0.093
Stoma problems	39.8 (20.0)	33.8 (20.3)	0.336	48.1 (31.7)	38.8 (16.9)	23.1 (20.9)	0.183
Flatulences	29.0 (38.5)	32.4 (35.2)	0.452	36.6 (41.7)	33.6 (37.8)	20.5 (31.1)	0.142
Faecal incontinence	23.4 (35.8)	29.4 (39.1)	0.391	40.0 (44.0)	28.7 (38.3)	14.5 (28.4)	0.050
Sore skin around anus	17.3 (29.6)	23.9 (33.9)	0.300	26.6 (36.8)	21.7 (32.8)	12.8 (24.9)	0.266
Stool frequency	27.8 (30.2)	31.8 (34.7)	0.836	35.8 (34.7)	33.0 (33.7)	18.3 (25.0)	0.029
Embarrassment	27.3 (36.5)	35.8 (40.4)	0.179	41.6 (38.8)	36.0 (40.8)	14.5 (26.2)	0.005
Defecation pb	26.9 (25.3)	30.9 (26.4)	0.499	37.5 (33.0)	30.3 (25.5)	17.5 (19.0)	0.032
Sexual function: Male				33.3 (36.9)	42.7 (37.8)	45.8 (36.5)	0.575
Impotence				48.7 (44.3)	35.2 (38.5)	42.0 (35.1)	0.465
Sexual function: Female				57.5 (36.7)	63.5 (37.7)	82.3 (31.4)	0.109
Dyspareunia				33.3 (36.5)	27.6 (36.8)	9.8 (22.8)	0.098

ARTICLE HIGHLIGHTS

Research background

Health-related quality of life is an abstract and multidimensional concept which can be assessed by the European Organization for Research and Treatment of Cancer (EORTC) questionnaires. Core measurement tools examine issues common to different cancer sites and can be used as a stand-alone questionnaire or in combination with disease specific modules.

Research motivation

The EORTC Quality of Life Questionnaire (QLQ) CR29 questionnaire specific to colorectal cancer (CRC) and its psychometric properties have been tested in several languages and contexts. Recently, The QLQ-CR29 has just been only translated for Moroccan Arabic dialect. However this adaptation was performed on a very limited sample size of 120 patients under the usual requests of the EORTC organization.

Research objectives
The aim of this study is to externally validate this version and assess its psychometric properties on larger Moroccan CRC patients.

Research methods
In order to externally validate the QLQ CR-29, Both Moroccan Arabic modules of QLQ CR-29 and QLQ C-30 were administered to Moroccan colorectal cancer (CRC). Psychometric properties were retested by measuring Cronbach's alpha coefficient for reliability and Intraclass correlation coefficient (ICC) to examine test-retest reproducibility. The multitrait-scaling analysis was performed to demonstrate the validity of the instrument and known-groups comparison was used to test the score's ability to discriminate between different groups of patients. All statistical analyses were performed using SPSS 26.0 (SPSS Inc., Chicago,IL, USA). Statistically significant results were defined with a $P < 0.05$.

Research results
In total, 221 patients were included in the study and 34 patients completed the questionnaire twice. The urinary Frequency scale and Stool Frequency scale had good internal consistency with alpha Cronbach coefficients of 0.79 and 0.83 respectively, while the same coefficients were moderately lower for the Blood and Mucus in Stool scale (0.61) and the Body Image scale (0.67). The ICCs ranged from 0.88 to 1 indicating good to excellent reproducibility. In multitrait scaling analyses, the criterion for item convergent and divergent validity was satisfactory. The known-group comparison showed statistically significant differences between patients according to age, gender, stoma status, tumor location, and radiotherapy.

Research conclusions
The Moroccan Arabic version of the EORTC QLQ-CR29 is a valid and reliable tool that can be used safely for research and clinical purposes in Moroccan CRC patients.

Research perspectives
This tool can safely be used in research and clinical purpose and can be also used in the validation of other patient-reported outcome measure tools.

FOOTNOTES

Author contributions: Souadka A and Bachri H have contributed to the conception and design of the study, acquisition of the data, the analysis and the interpretation of data; Souadka A, Bachri H and Essangri H wrote the first draft; El Bahaoui N, Majbar AM and Benkabbou A critically reviewed the draft for important intellectual content; Mohsine R was involved in revising critically the corrected manuscript and all authors read and gave the final approval of the version to be published.

Institutional review board statement: The Approval of the study protocol was obtained from the institutional ethics review board (number: 79/17) and all patients enrolled in the study provided a written, informed consent allowing the use of their data for clinical studies at the time of their initial visit.

Conflict-of-interest statement: The authors declare no conflict of interest.

Data sharing statement: Derived data supporting the findings of this study are available from the corresponding author a.souadka@um5r.ac.ma upon reasonable request.

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Country/Territory of origin: Morocco

ORCID number: Hajar Essangri 0000-0001-8218-3602; Amine Benkabbou 0000-0003-2769-1115; Raouf Mohsine 0000-0001-8590-9333; Anass Mohammed Majbar 0000-0003-4329-2026; Amine Souadka 0000-0002-1091-9446.

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ORIGINAL ARTICLE

Observational Study

Biliary fistula and late recurrence of liver hydatid cyst: Role of cysto-biliary communication: A prospective multicenter study

Tamer A A M Habeeb, Mauro Podda, Boris Tadic, Vishal G Shelat, Yaman Tokat, Mohamed Ibrahim Abo Alsaad, Abd-Elfattah Kalmoush, Mohammed Shaaban Nassar, Fawzy Metwally Mustafa, Mahmoud Hassib Morsi Badawy, Mohamed Sobhy Shaaban, Tarek Zaghloul Mohamed, Mohammed Ibrahim El Sayed Henish, Hamdi Elbelkasi, Mahmoud Abdou Yassin, Abdelshafy Mostafa, Amr Ibrahim, Waleed A-Abdelhady, Tamer Mohamed Elshahidy, Mohamed Ibrahim Mansour, Adel Mahmoud Moursi, Mohamed Abdallah Zaitoun, Ehab Shehata Abd-Allah, Ashraf Abdelmonem Elsayed, Rasha S Elsayed, Ahmed M Yehia, Amr Abdelghani, Mohamed Negm, Heba Alhussein Abo-Alella, Mostafa M Elaidy

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Tamer A A M Habeeb, Mahmoud Abdou Yassin, Abdelshafy Mostafa, Amr Ibrahim, Waleed A-Abdelhady, Tamer Mohamed Elshahidy, Mohamed Ibrahim Mansour, Adel Mahmoud Moursi, Mohamed Abdallah Zaitoun, Ehab Shehata Abd-Allah, Ashraf Abdelmonem Elsayed, Rasha S Elsayed, Ahmed M Yehia, Amr Abdelghani, Mohamed Negm, Heba Alhussein Abo-Alella, Mostafa M Elaidy, Department of General Surgery, Faculty of Medicine Zagazig University, Sharkia 44511, Egypt

Mauro Podda, Department of Surgical Science, University of Cagliari, Cagliari 09126, Italy

Boris Tadic, Clinic for Digestive Surgery – First Surgical Clinic, University Clinical Centre of Serbia, Belgrade 11000, Serbia

Vishal G Shelat, Department of General Surgery, Tan Tock Seng Hospital, Singapore 018971, Singapore

Yaman Tokat, Department of Liver Transplantation and Hepatobiliary Surgery, Istanbul, Turkey, International Liver Center, and Acibadem Hospital Group, Istanbul 34000, Turkey

Mohamed Ibrahim Abo Alsaad, Department of General Surgery, Faculty of Medicine -Merit University, Sohag 82511, Egypt

Abd-Elfattah Kalmoush, Mohammed Shaaban Nassar, Fawzy Metwally Mustafa, Mahmoud Hassib Morsi Badawy, Mohamed Sobhy Shaaban, Tarek Zaghloul Mohamed, Mohammed Ibrahim El Sayed Henish, Department of General Surgery, Faculty of Medicine, Al-Azher University, Cairo 11765, Egypt

Hamdi Elbelkasi, Department of General Surgery, Mataryia Teaching Hospital, Cairo 11765, Egypt

Corresponding author: Tamer A A M Habeeb, MD, Professor, Department of General Surgery, Faculty of Medicine Zagazig University, Sharkia 44511, Egypt. tameralnaimy@hotmail.com

Abstract

BACKGROUND

Hydatid cyst disease (HCD) is common in certain locations. Surgery is associated with postoperative biliary fistula (POBF) and recurrence. The primary aim of this study was to identify whether occult cysto-biliary communication (CBC) can predict recurrent HCD. The secondary aim was to assess the role of cystic fluid bilirubin and alkaline phosphatase (ALP) levels in predicting POBF and recurrent HCD.

AIM

To identify whether occult CBC can predict recurrent HCD. The secondary aim was to assess the role of cystic fluid bilirubin and ALP levels in predicting POBF and recurrent HCD.

METHODS

From September 2010 to September 2016, a prospective multicenter study was undertaken involving 244 patients with solitary primary superficial stage cystic echinococcosis 2 and cystic echinococcosis 3b HCD who underwent laparoscopic partial cystectomy with omentoplasty. Univariable logistic regression analysis assessed independent factors determining biliary complications and recurrence.

RESULTS

There was a highly statistically significant association ($P \leq 0.001$) between cystic fluid biochemical indices and the development of biliary complications (of 16 patients with POBF, 15 patients had high cyst fluid bilirubin and ALP levels), where patients with high bilirubin-ALP levels were 3405 times more likely to have biliary complications. There was a highly statistically significant association ($P \leq 0.001$) between biliary complications, biochemical indices, and the occurrence of recurrent HCD (of 30 patients with recurrent HCD, 15 patients had high cyst fluid bilirubin and ALP; all 16 patients who had POBF later developed recurrent HCD), where patients who developed biliary complications and high bilirubin-ALP were 244.6 and 214 times more likely to have recurrent hydatid cysts, respectively.

CONCLUSION

Occult CBC can predict recurrent HCD. Elevated cyst fluid bilirubin and ALP levels predicted POBF and recurrent HCD.

Key Words: Cysto-biliary communication; *Echinococcus granulosus*; Hydatid disease recurrence; Hydatid fluid analysis; Laparoscopy

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Core Tip: There has been no research on occult cysto-biliary communication (CBC) prediction for recurrent hydatid cyst disease (HCD) or the role of elevated cyst fluid bilirubin and alkaline phosphatase (ALP) levels in predicting postoperative biliary fistula and recurrent HCD. The main finding of this study was that there was a statistically significant association ($P \leq 0.001$) between biochemical indices and the development of biliary complications, where patients with high bilirubin-ALP levels were 3405 times more likely to have biliary complications. There was a highly statistically significant association between biliary complications, biochemical indices, and the occurrence of recurrent HCD, where patients who developed biliary complications and high bilirubin-ALP were 244.6 and 214 times more likely to have recurrent hydatid cysts, respectively.

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INTRODUCTION

Hydatid cyst disease (HCD) is caused by *Echinococcus granulosus*, which infects humans in many areas worldwide. Different organs may be affected by HCD[1-2]; however, the liver is mainly affected by HCD (50%-70%)[3]. The World Health Organization's Informal Working Group on Echinococcosis (WHO-IWGE) advocated a standardized consensus in diagnosis, treatment, and follow-up[4].

Medical treatment alone is ineffective, whereas laparoscopic or open surgical management of HCD is an integral treatment component[5-6]. With advances in minimal access surgery, laparoscopic HCD surgery is increasingly being performed, varying from the more conservative choice of partial cystectomy and omentoplasty to the more radical choice of pericystectomy or hepatic resection determined by cyst location, cyst size, risk of complications, and surgical expertise [7]. As HCD is benign, liver resections are avoided as much as possible to reduce perioperative morbidity, such as liver failure[8]. Although a case report used the Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy procedure, it has not yet been demonstrated[9].

Cysto-biliary communication (CBC), which presents as a postoperative biliary fistula (POBF), is a common postoperative complication of hepatic HCD, with an incidence of 37%. CBC may be occult or manifest with various clinical symptoms. In occult CBC, cyst fluid, scolices, small daughter cysts, and laminated and germinal layer fragments migrate into the biliary tract and remain asymptomatic or undetectable on imaging. If an occult CBC is missed during surgery, POBF manifests as an external biliary fistula, biliary peritonitis, or intra-abdominal abscess[10-11]. Another concern with HCD surgery is recurrent HCD, which occurs in 11% of cases[12]. The primary aim of this study was to determine whether occult CBC, expressed as POBF, is associated with recurrent HCD. The secondary aim was to determine whether elevated cyst fluid bilirubin and alkaline phosphatase (ALP) levels predict occult CBC and, thus, POBF and recurrent HCD.

MATERIALS AND METHODS

Study design and participants

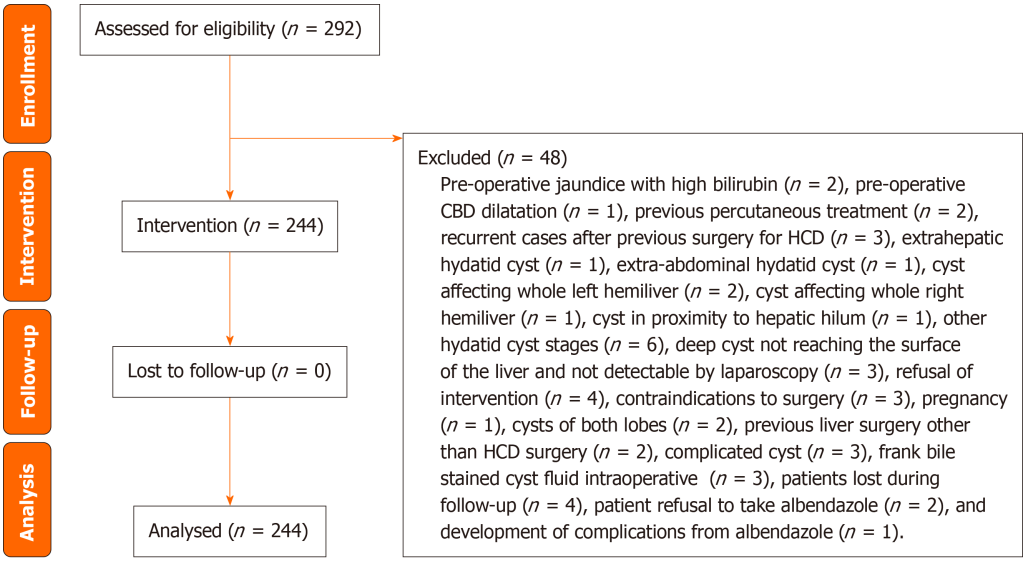
From September 2010 to September 2016, a prospective observational multicenter study was carried out at our university hospitals involving 244 patients with stages cystic echinococcosis 2 (CE2) and cystic echinococcosis 3b (CE3b) according to the WHO-IWGE classification. Patients with large, solitary, symptomatic, or asymptomatic superficial cysts abutting critical vascular and biliary structures were included in this study. Exclusion criteria were: (1) Stage CE1, CE3a, and CE4 HCD patients (WHO- IWGE recommendation); (2) preoperative factors: Total bilirubin > 2.0 mg/dL and direct bilirubin > 1.5 mg/dL; (3) prior liver interventions: Previous percutaneous treatment, recurrence of HCD, previous liver surgery; (4) radiological factors: Common bile duct (CBD) dilatation > 10 mm, extrahepatic cysts affecting an entire hemiliver, complicated (ruptured) or deep location that cannot be accessed by laparoscopy and hydatid cysts in both lobes of the liver; (5) patient factors: Referenceused intervention, surgical contraindication, pregnancy, lost to follow-up, refused to take albendazole, or patients who had albendazole-related complications (deranged liver function); and (6) intraoperative factors: Manifest CBC. This study was approved by the Institutional Review Board committees and registered as a clinical trial (ClinicalTrials.gov ID: NCT05116735) and was conducted following the Helsinki Declaration and STROBE[13].

Definitions of outcomes and measurements

POBF was defined as a bilirubin concentration in the drain fluid at least three times higher than the serum bilirubin concentration on or after postoperative day three[14]. Hydatid recurrence was defined as the appearance of new active cysts after surgery[15]. Postoperative residual fluid cavities were not classified as recurrent HCD[12]. Postoperative morbidity was assessed using the Clavien-Dindo classification[16]. Hydatid cyst fluid bilirubin and ALP levels were measured using an automatic biochemical analyzer (AU-400, Olympus)[17]. All hospitals used the same instrument to measure bilirubin and ALP levels in the cyst fluid. All the machines were calibrated at each institution to ensure consistent reporting. The time from surgery to disease relapse at any site was described as recurrence-free survival.

Perioperative procedure and follow-up

A multidisciplinary team, including surgical, radiological, and anesthetic specialists, preoperatively evaluated all patients. Abdominal ultrasound, contrast-enhanced computed tomography (CT), and magnetic resonance cholangiopancreatography were performed at the discretion of the leading surgeon[6]. The protocol therapy in our hospitals for



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Figure 1 Flow diagram of the inclusion and exclusion criteria. CBD: Common bile duct; HCD: Hydatid cyst disease.

albendazole was 10 mg/kg ten days before surgery and continued for six months following surgery with a two-week interval between each month. Complete blood counts and liver function monitoring were routinely performed. The surgical technique has been previously reported in detail[18]. In short, pneumoperitoneum with an intra-abdominal pressure of 14 mmHg was established. Fine needle aspiration of the cystic fluid was performed to rule out manifest CBC. The aspirated cyst fluid was analyzed for fluid bilirubin and ALP levels before injection of hypertonic saline, and the bilirubin and ALP levels were determined after surgery. The cystic wall was partially removed using a harmonic scalpel. To detect occult CBC, after the cystic cavity was fully evacuated and complete hemostasis was achieved, the intra-abdominal pressure was lowered to 10 mmHg. Visible orifices of the CBC were searched by thorough laparoscopic exploration of the cyst cavity; 20% hypertonic saline-soaked gauze was placed in the cystic cavity to observe the presence of bile while compressing either the gallbladder or CBD to stimulate bile backflow through the CBC (if present, the CBC was sutured).

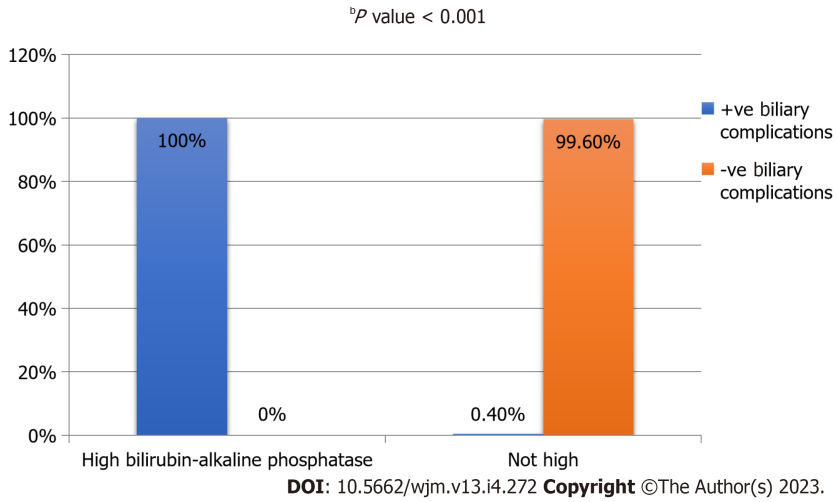
Furthermore, we injected air, saline, or methylene blue dye in succession in all patients *via* cystic duct cannulation with CBD occlusion, hoping to increase CBC detection rates (three cases changed from occult CBC to manifest CBC intraoperatively and were excluded from the study). Omentoplasty was performed by inserting a right gastroepiploic-based omental flap into the cavity. A drain was placed in the subhepatic area. After three days, the drain was removed if there was no evidence of bile leakage. The patients were discharged on the fifth postoperative day. Follow-up visits were scheduled after one month, three months, six months, one year, and then every six months for the next four years. The drainage tube was left longer until fistula resolution was achieved in patients with bile leaks. After surgery, an ultrasound scan was used to check the cyst cavity at one, three, six months, one year, and then every six months until completion of the follow-up period (5 years). If the ultrasound results were inconclusive, a CT scan was performed. No mortality was observed in this study.

Statistical analysis

Statistical Package for Social Science software was used to analyze the collected data (version 20.0. IBM Corp., Armonk, NY, USA). Continuous variables with a normal distribution were reported as mean and SD, while data with a non-normal distribution were presented as medians and ranges. Absolute and relative frequencies were used to summarize categorical variables. The Chi-square test (χ^2) or Fisher's exact test was used to examine categorical variables. Independent factors determining biliary complications and recurrence were analyzed using univariate logistic regression analysis. The odds ratio (OR) was calculated to compare the relative odds of biliary complications and recurrence of HCD. The 95%CI was used to estimate OR precision. The hydatid cyst-recurrence-free time was determined using the Kaplan–Meier method. Statistically significant and highly statistically significant variables were defined as P values < 0.05 and < 0.001, respectively.

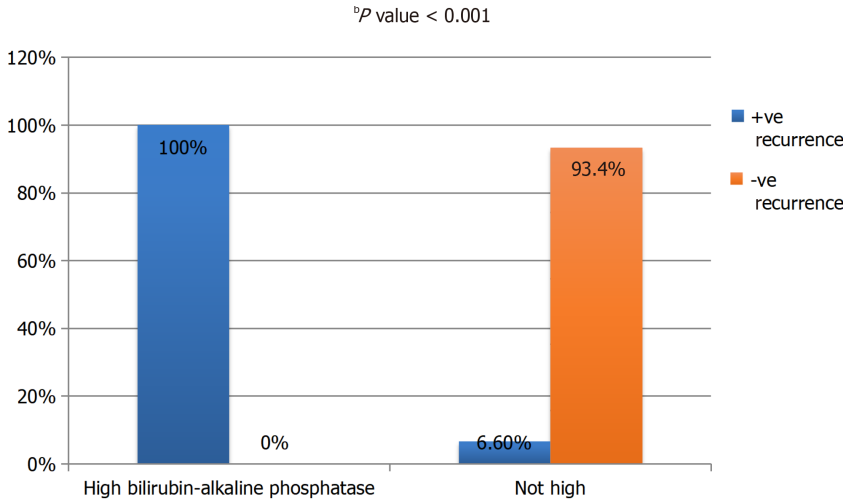
RESULTS

A flowchart of the eligibility criteria is presented in Figure 1. A total of 292 patients with hepatic HCD were referred to our clinics based on abdominal ultrasound results. Forty-eight patients were excluded: Pre-operative jaundice with high bilirubin (n = 2), pre-operative CBD dilatation (n = 1), previous percutaneous treatment (n = 2), recurrent cases after previous surgery for HCD (n = 3), extrahepatic hydatid cyst (n = 1), extra-abdominal hydatid cyst (n = 1), cyst affecting whole left hemiliver (n = 2), cyst affecting whole right hemiliver (n = 1), cyst in proximity to hepatic hilum (n = 1), other



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Figure 2 Relation between the biochemical indices and development of biliary complications among the studied patients (*n* = 244). ^{b}P < 0.01. + ve: Presence of biliary complications; - ve: Absence of biliary complications.



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Figure 3 Relation between the biochemical indices and recurrence of hydatid cyst among the studied patients (*n* = 244). ^{b}P < 0.01. + ve: Occurrence of recurrence; - ve: No recurrence.

hydatid cyst stages (*n* = 6), deep cyst not reaching the surface of the liver and could not be detected by laparoscopy (*n* = 3), refused intervention (*n* = 4), contraindications to surgery (*n* = 3), pregnancy (*n* = 1), cysts of both lobes (*n* = 2), previous liver surgery other than HCD surgery (*n* = 2), complicated cyst (*n* = 3), intraoperative frank bile stained cyst fluid (*n* = 3), patients lost during follow-up (*n* = 4), patient refusal to take albendazole (*n* = 2), and development of complications due to albendazole (*n* = 1). Following the exclusion of 48 patients, a total of 244 patients were enrolled in this study.

The patients' demographic and preoperative characteristics are summarized in Table 1. This study included 244 patients with a mean age of 42.67 ± 9.97 years. The mean cyst diameter was 9.49 ± 3.54 cm, and the most common symptom was abdominal pain (*n* = 125, 51.2%). Most patients had a cyst classification of CE3b (*n* = 183, 75%) with a predominant right hemiliver location (*n* = 184, 75.4%).

Table 2, Figures 2 and 3 show the intraoperative and postoperative findings, respectively. The mean operative time was 107.32 ± 6.92 min, with cystic fluid spilling (4.9%) and high bilirubin-high ALP in cyst fluid (6.1%). Biliary complications occurred in 16 patients (6.6%), and recurrent HCD occurred in 30 patients (12.3%).

Table 3 shows that the mean time to biliary fistula detection was 3.13 ± 1.78 d, and the resolution of bile leak was 14.38 ± 6.12 d. Patients with POBF (*n* = 16/244, 6.6%) had mainly external biliary fistula (*n* = 9/16, 56.2%). Most cases were grade B (7/16, 43.7%). The mean time to recurrent HCD was 2.48 ± 0.98 years with a cyst size of 4.93 ± 2.72 cm. Recurrent HCD was most common in the right hemiliver (18/30, 60%) and with CE2 as the cyst stage (25/30, 83.3%). There was no mortality in our study. A highly statistically significant association ($P \leq 0.001$) was observed between biochemical indices and the development of biliary complications, where patients with high bilirubin-ALP were 3405 times more likely to have biliary complications (Table 4). There was a highly statistically significant association ($P \leq 0.001$) between biliary complications, biochemical indices, and the occurrence of recurrence HCD where patients who developed biliary complications and high bilirubin-ALP were 244.6 and 214 times more likely to have recurrent hydatid cyst, respectively

Table 1 Demographic and preoperative characteristics of the patients (*n* = 244), *n* (%)

Characteristics	*n* = 244
Age (year) mean ± SD	42.67 ± 9.97
≤ Median (43)	136 (55.7)
> Median (43)	108 (44.3)
Sex	
Male	163 (66.8)
Female	81 (33.2)
ASA	
I	176 (72.1)
II	47 (19.3)
III	21 (8.6)
Positive family history	15 (6.1)
Clinical presentation	
Asymptomatic	19 (7.8)
Pain	125 (51.2)
Mass	48 (19.7)
Nausea & vomiting	32 (13.1)
Dyspepsia	20 (8.2)
Cyst size (cm) mean ± SD	9.49 ± 3.54
≤ Median (9)	130 (53.3)
> Median (9)	114 (46.7)
Cyst site	
Right lobe	184 (75.4)
Left lobe	60 (24.6)
Cyst stage	
CE2	61 (25)
CE3b	183 (75)

ASA: American Society of Anesthesiologists; CE: Cystic echinococcosis.

(Table 5, Figure 4).

DISCUSSION

This study evaluated the association between occult CBC, POBF, and recurrent HCD. The study results further highlight the importance of hydatid cyst fluid analysis in detecting occult CBC. To the best of our knowledge, no research published in the literature has addressed the relationship between occult CBC and the development of recurrent HCD or the relationship between elevated cyst fluid bilirubin and ALP and the development of POBF and recurrent HCD. This study showed that occult CBC occurred in 6.6% of patients and recurrent HCD occurred in 12.3% of patients. Of the 16 patients with POBF, 15 had high cyst fluid bilirubin and ALP levels, and patients with high bilirubin-ALP were 3405 times more likely to have biliary complications. Of the 30 patients with recurrent HCD, 15 patients had high cyst fluid bilirubin and ALP levels; all 16 patients with POBF later developed recurrent HCD, and patients who developed biliary complications and high bilirubin-ALP were 244.6 and 214 times more likely to have recurrent hydatid cysts, respectively. The detection of occult CBC is essential. In our study, occult CBC and the subsequent development of postoperative biliary leak occurred in 16 patients (6.6%), which was lower than that in the two studies (27% and 16%, respectively)[19-20]. The low incidence of POBF in our study may be due to variations in sample size, different surgical approaches, and inclusion criteria, especially deep/centrally located cysts, such as those in medial segments such as IVa, V, and VIII, extended deep into the liver parenchyma and was closely related to major biliovascular structures with a higher incidence

Table 2 Intraoperative and postoperative outcomes (*n* = 244), *n* (%)

Intraoperative outcome	*n* = 244
Operative time (min) (mean ± SD)	107.32 ± 6.92
≤ Median (106)	126 (51.6)
> Median (106)	118 (48.4)
Blood transfusion	
1 unit	4 (1.6)
2 units	2 (0.8)
Cystic fluid spillage	12 (4.9)
Conversion	7 (2.9)
Causes of conversion	
Extensive adhesion	5 (2.1)
Organ injury	1 (0.4)
Difficult cyst detection	1 (0.4)
Anaphylaxis	2 (0.8)
Cyst fluid biochemical indices	
High both bilirubin-high alkaline phosphatase	15 (6.1)
Postoperative outcomes	*n* = 244
Postoperative hospital stay (days) mean ± SD	3.44 ± 2.51, 3 (2-20)
ICU admission	9 (3.7)
Early postoperative complications	28 (11.5)
Type of early postoperative complications	
Wound infection	7 (2.9)
Cyst cavity bile collection	6 (2.5)
Ileus	2 (0.8)
Subphrenic abscess	4 (1.6)
Subphrenic hematoma	3 (1.2)
Cholangitis	4 (1.6)
Pneumonia	1 (0.4)
Atelectasis	1 (0.4)
None	215 (88.1)
Clavien-Dindo classification	
0	213 (87.3)
I	8 (3.3)
II	11 (4.5)
III	12 (4.9)
Biliary complications	16 (6.6)
Late postoperative complications	
Port/incisional site hernia	7 (2.9)
Recurrence	30 (12.3)

ICU: Intensive care unit.

Table 3 Characteristics of biliary complications and recurrence (*N* = 16), *n* (%)

Biliary complications	*n* = 16
Type	
External biliary fistula	9 (56.2)
Cyst cavity biliary abscess	4 (25)
Biliary peritonitis	3 (18.8)
Grade	
Grade A	6 (37.5)
Grade B	7 (43.7)
Grade C	3 (18.8)
Time of development (days) mean ± SD	3.13 ± 1.78
Treatment	
Conservative	6 (37.5)
Ultrasound guided percutaneous drainage	7 (43.7)
Reoperation	3 (18.8)
Time to leakage cessation (days) mean ± SD	14.38 ± 6.12
Recurrence	*n* = 30
Site	
Right lobe	18 (60)
Left lobe	6 (20)
Peritoneum	4 (13.3)
Spleen	2 (6.7)
Clinical presentation	
Asymptomatic	7 (23.3)
Pain	13 (43.3)
Mass	4 (13.3)
Nausea & vomiting	2 (6.7)
Infection	2 (6.7)
Rupture	1 (3.3)
Jaundice	1 (3.3)
Time to diagnosis (yr) mean ± SD	2.48 ± 0.98
Size (cm) mean ± SD	4.93 ± 2.72
Stage	
CE2	25 (83.3)
CE3b	5 (16.7)
Treatment	
Pericystectomy	17 (56.7)
Left hepatectomy	6 (20)
Abdominal exploration and excision of recurrent abdominal HCD	4 (13.3)
Splenectomy (for recurrent splenic HCD)	2 (6.7)
CBD stent + pericystectomy	1 (3.3)

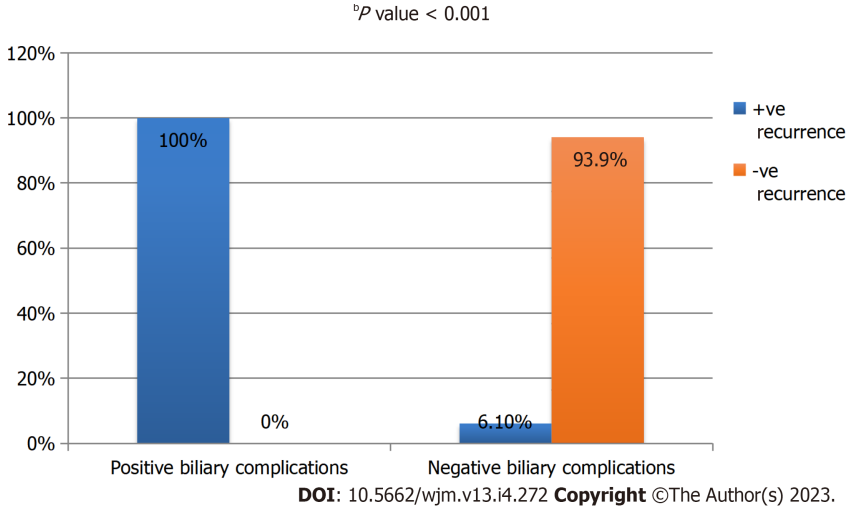
CE: Cystic echinococcosis; CBD: Common bile duct; HCD: Hydatid cyst disease.

Table 4 Relation between the independent factors and development of biliary complications among the studied patients by univariate analysis (*n* = 244)

Factors	Biliary complications (*n* = 16)		No biliary complications (*n* = 228)		*P* value	Univariate OR (95%CI)
	n	%	*n*	%		
Sex						
Male (*n* = 163)	9	5.5	154	94.5	0.354[1]	Reference
female (*n* = 81)	7	8.6	74	91.4		1.62 (0.58-4.52)
ASA						
I (*n* = 176)	9	5.1	167	94.9	0.160[1]	1.08 (0.12-8.96)
II (*n* = 47)	6	12.8	41	87.2		2.93 (0.33-25.98)
III (*n* = 21)	1	4.8	20	95.2		Reference
Family history						
+ ve (*n* = 15)	8	53.3	7	46.7	< 0.001[2,b]	31.6 (9.18-108.6)
- ve (*n* = 229)	8	3.5	221	96.5		Reference
Cyst size (cm)						
≤ 9 cm (*n* = 130)	3	2.3	127	97.9	0.004[1,a]	Reference
> 9 cm (*n* = 114)	13	11.4	101	88.6		5.45 (1.51-19.64)
Cyst site						
Right lobe (*n* = 184)	13	7.1	171	92.9	0.575[2]	1.44 (0.39-5.25)
Left lobe (*n* = 60)	3	5	57	95		Reference
Cyst stage						
CE2 (*n* = 61)	15	24.6	46	75.4	< 0.001[2,b]	59.3 (7.64-460.9)
CE3b (*n* = 183)	1	0.5	182	99.5		Reference
Operative time (min)						
≤ 106 min (*n* = 126)	9	7.1	117	92.9	0.703[1]	1.22 (0.44-3.39)
> 106 min (*n* = 118)	7	5.9	111	94.1		Reference
Blood transfusion						
Yes (*n* = 6)	4	66.7	2	33.3	< 0.001[2,b]	37.7 (2.26-226.5)
No (*n* = 238)	12	5	226	95		Reference
Cystic fluid spillage						
Yes (*n* = 12)	2	16.7	10	83.3	0.147[2]	3.11 (0.62-15.60)
No (*n* = 232)	14	6	218	94		Reference
Conversion						
Yes (*n* = 7)	5	71.4	2	28.6	< 0.001[2,b]	51.4 (8.94-294.9)
No (*n* = 237)	11	4.6	226	95.4		Reference
Anaphylaxis						
Yes (*n* = 2)	0	0	2	100	0.707[1]	Reference
No (*n* = 244)	16	6.6	226	93.4		0.14 (0.01-1.65)
Cyst fluid biochemical indices						
High bilirubin-alkaline phosphatase (*n* = 15)	15	100	0	0.0	< 0.001[2,b]	3405 (202-57164)
Not high (*n* = 229)	1	0.4	228	99.6		Reference
Hospital stay (days)						

≤ 3 d (*n* = 184)	0	0.0	184	100	< 0.001[2,b]	Reference
> 3 d (*n* = 60)	16	26.7	44	73.3		67 (8.64-518.15)
ICU admission						
Yes (*n* = 9)	5	55.6	4	44.4	< 0.001[2,b]	25.5 (5.99-108.24)
No (*n* = 235)	11	4.7	224	95.3		Reference
Early postoperative complications						
Yes (*n* = 28)	13	46.4	15	53.6	< 0.001[2,b]	61.5 (15.8-239.8)
No (*n* = 216)	3	1.4	213	98.6		Reference

[1]Chi square test (χ^2).
[2]Fisher's exact test.
[a]*P* ≤ 0.05: Statistically significant.
[b]*P* ≤ 0.001: Highly statistically significant.
OR: Odds Ratio; ICU: Intensive care unit, ASA: American Society of Anesthesiologists; CE: Cystic echinococcosis; + ve: Positive family history; - ve: Negative family history.



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Figure 4 Relation between the biliary complications and recurrence of hydatid cyst among the studied patients (*n* = 244). [b]*P* < 0.01. + ve: Occurrence of recurrence; - ve: Absence of recurrence.

of CBC in these studies. Furthermore, we meticulously searched for tiny CBC using various techniques. Another challenge during the laparoscopic surgical approach for liver HCD is that pneumoperitoneum increases intra-abdominal pressure and may prevent bile leakage into the cyst. We faced this challenge through adequate laparoscopic exploration of the cyst cavity after cyst deroofing, intraoperative dye injection, and white gauze left inside the residual cavity for a few minutes and lowering the intra-abdominal pressure to 10 mmHg, which could facilitate CBC detection. Ultimately, this helped decrease the incidence of POBF. Of the 16 patients who developed POBF, 15 (15/16) had high cyst fluid bilirubin and ALP levels. CBC is the cause of high levels of these biochemicals in cyst fluid. The high level of cyst fluid in the postoperative period led us to suspect the development of POBF in these patients. Another point of contention is the relationship between cyst size and the development of CBC. The relationship between cyst size and CBC varies between studies, with numerous studies reporting a cyst size cut-off of 10 cm for CBC[21-24]. We found a significant correlation between cyst size and POBF, with cysts larger than the median 9 cm being 5.45 times more likely to develop POBF. A larger cyst is more likely to deform the biliary pedicle anatomy owing to the greater exposed surface area, thus predisposing the patient to CBC. Therefore, size has a direct relationship with the CBC. Furthermore, it has been reported that intracystic pressure increases with the diameter of the hepatic cyst, increasing the likelihood of CBC[25]. In our experience, POBF occurred on the third postoperative day, mainly as an external biliary fistula (9/16, 56.2%). Most patients had grade B disease (7/16, 43.7%). Most POBFs were treated conservatively or with ultrasound-guided percutaneous drainage, and all patients demonstrated clinical resolution within two weeks. In our study, endoscopic biliary stenting was unnecessary except in patients with persistent bile leaks.

Recurrence of HCD is the second most common complication for both surgeons and patients. Failure to remove all viable cysts and protoscolices, particularly long-standing cysts with host tissue adventitial layer (pericyst) branching into the surrounding tissue, may be responsible for recurrence. Although the laparoscopic approach has many advantages during HCD surgery, drawbacks include difficulty in accessing a cyst, difficult aspiration with a fine needle that may be

Table 5 Relation between the independent factors and recurrence of hydatid cyst by univariate analysis (*n* = 244)

Factors	Recurrence (*n* = 30)		No recurrence (*n* = 214)		*P* value	Univariate OR (95%CI)
	n	%	*n*	%		
Sex						
Male (*n* = 163)	18	0.11	145	0.89	0.398[1]	Reference
Female (*n* = 81)	12	0.148	69	0.852		1.40 (0.62-3.07)
ASA						
I (*n* = 176)	21	0.119	155	0.881	0.792[1]	1.29 (0.28-5.924)
II (*n* = 47)	7	0.149	40	0.851		1.66 (0.31-8.78)
III (*n* = 21)	2	0.095	19	0.905		Reference
Family history						
+ve (*n* = 15)	15	1	0	0	< 0.001[2,b]	214 (26.45-1731.7)
-ve (*n* = 229)	15	0.066	214	0.934		Reference
Cyst size (cm)						
≤ 9 cm (*n* = 130)	7	0.054	123	0.946	< 0.001[1,b]	Reference
> 9 cm (*n* = 114)	23	0.202	91	0.798		4.44 (1.83-10.80)
Cyst site						
Right lobe (*n* = 184)	23	0.125	161	0.875	0.864[1]	1.08 (0.44-2.66)
Left lobe (*n* = 60)	7	0.117	53	0.883		Reference
Cyst stage						
CE2 (*n* = 61)	27	0.443	34	0.557	< 0.001[1,b]	47.7 (13.7-165.95)
CE3b (*n* = 183)	3	0.016	180	0.984		Reference
Operative time (min)						
≤ 106 min (*n* = 126)	16	0.127	110	0.873	0.843[1]	1.08 (0.50-2.324)
> 106 min (*n* = 118)	14	0.119	104	0.881		Reference
Blood transfusion						
Yes (*n* = 6)	5	0.833	1	0.167	< 0.001[2,b]	42.6 (4.78-397.4)
No (*n* = 238)	25	0.105	213	0.895		Reference
Cystic fluid spillage						
Yes (*n* = 12)	12	1	0	0	< 0.001[2,b]	142 (17.5-1160.4)
No (*n* = 232)	18	0.078	214	0.922		Reference
Conversion						
Yes (*n* = 7)	6	0.857	1	0.143	< 0.001[2,b]	53.3 (6.15-461.2)
No (*n* = 237)	24	0.101	213	0.899		Reference
Anaphylaxis						
Yes (*n* = 2)	2	1	0	0	< 0.001[2,b]	15.3 (1.34-174.1)
No (*n* = 244)	28	0.116	214	0.884		Reference
Cyst fluid biochemical indices						
High bilirubin-alkaline phosphatase (*n* = 15)	15	1	0	0	< 0.001[2,b]	214 (26.45-1731.7)
Not high (*n* = 229)	15	0.066	214	0.934		Reference
Hospital stay (days)						
≤ 3 d (*n* = 184)	10	0.054	174	0.946	< 0.001[1,b]	Reference
> 3 d (*n* = 60)	20	0.333	40	0.667		8.7 (3.78-20.02)

ICU admission						
Yes (n = 9)	8	0.889	1	0.111	< 0.001[2,b]	77.5 (9.25-648.4)
No (n = 235)	22	0.094	213	0.906		Reference
Early postoperative complications						
Yes (n = 28)	22	0.786	6	0.214	< 0.001[2,b]	95.33 (30.3-299.9)
No (n = 216)	8	0.037	208	0.963		Reference
Biliary complications						
Yes (n = 16)	16	1	0	0	< 0.001[2,b]	244.6 (30.21-1981)
No (n = 228)	14	0.061	214	0.939		Reference
Port/incisional site hernia						
Yes (n = 7)	7	1	0	0	< 0.001[2,b]	65.13 (7.67-553.1)
No (n = 237)	23	0.097	214	0.903		Reference

[1]Chi square test (χ^2).
[2]Fisher's exact test.
[a]$P \leq 0.05$: Statistically significant.
[b]$P \leq 0.001$: Highly statistically significant.
OR: Odds Ratio; ICU: Intensive care unit; ASA: American Society of Anesthesiologists; CE: Cystic echinococcosis; + ve: Positive family history; - ve: Negative family history.

obstructed by fragments of the laminated and germinal layers, and puncture of the cyst under pressure with an associated risk of intraoperative spillage and thus recurrence that may reach up to 22%[26-27]. According to our results, 12.3% of the patients developed recurrent HCD, which was lower than in two previous reports. This was due to differences in technique, cyst size, or follow-up duration. We routinely release the pneumoperitoneum to reduce intra-abdominal pressure during puncture and aspiration of the cyst, aiming to decrease intra-abdominal spillage. However, unrecognized spillage of cyst contents due to multiple cyst punctures by fine needles could contribute to spillage and dissemination, which may contribute to recurrence. In our opinion, the larger sample size, intense and longer follow-up protocol in our study is a true reflection of the real recurrence risk. The proposed solutions to these recurrence challenges have been developed by Bickel *et al*[28] and Palanivelu *et al*[29]. These two previous studies did not report recurrence, as both provided contamination-free management of HCD with a large-diameter suction needle and suction apparatus that allowed cyst aspiration without multiple punctures.

According to our results, 12.3% of patients developed recurrent HCD, higher than in two previous reports. This was due to differences in technique, cyst size, or follow-up duration. The follow-up period for Bickel *et al*[28] was 4.1 years, and for Palanivelu *et al*[29], was 5.8 years. In our study, the mean duration to diagnosis of recurrent HCD was 2.48 ± 0.98 years, and the mean cyst size at recurrence was 4.93 ± 2.72 cm. The recurrent cyst size was less than the original preoperative cyst size, most likely due to regular follow-up, and thus cysts were detected earlier before becoming symptomatic. Ultrasound scans were primarily used to detect recurrent HCD during the follow-up period. CT was performed in patients with inconclusive ultrasound results. The diagnostic accuracies of ultrasonography and CT scans were 90% and 100%, respectively, and we did not rely on routine serological testing owing to its low specificity[30-31]. Occult CBC with POBF indicates cyst invasion and, consequently, recurrence. Our data showed that POBF was strongly associated with recurrence (all patients with POBF later developed recurrent HCD), possibly due to the laminated layer and germinal layer invasion into the surrounding liver tissue or biliary tree. Hydatid cyst generally is an "expansive" disease, not "infiltrative." However, with growth in size, distorted anatomy, and elevated intracystic pressure, the adjacent liver parenchyma may develop pressure necrosis. CBC can lead to POBF, which, if persistent, can cause the formation of cystic recurrence, particularly starting from the fragment of cyst lying on the biliary and vascular structure that was not removed during the operation. We equate the management of HCD with oncologic principles: (1) Multidisciplinary management; (2) treatment with intent to cure; (3) active monitoring for recurrence; and (4) long duration of follow-up beyond five years.

Regarding recurrent HCD treatment, there is consensus that non-surgical treatment is preferable for small asymptomatic cysts in elderly patients with comorbidities. The surgical approach is preferred for patients with large, symptomatic, and complicated cysts[32]. However, in our study, radical surgery was recommended unless contraindicated. In our experience, a patient with a predicted life expectancy of 5-10 years should be offered surgery. Informed decision-making is essential because once a decision is made for nonoperative watchful waiting, the clock does not stop. There is a possibility that the cyst may expand, leading to complications, and surgery may become more complicated with an increased risk of morbidity. Thus, early and timely surgery in patients with recurrent small HCD may be advantageous. The most common re-operative procedures in our experience were open pericystectomy (56.7%) and open left hepatectomy (20%). Laparoscopic splenectomy was performed for recurrent HCD of the spleen. Laparoscopic excision was performed in patients with recurrent intraperitoneal HCD.

The limitation of this study is that we omitted patients with multiple or recurrent HCD and those managed with open surgery. In addition, we did not perform a multivariate analysis due to the small number of patients with CBC and recurrent HCD. The low incidence of complications namely POBF and recurrence constitutes a limitation for conducting a reliable regression analysis to predict these outcomes. It may compromise the statistical power and validity of the model, impeding the identification of significant predictors and the generalization of the results to larger populations. Despite these limitations, the information presented in this study adds to the current body of scientific evidence, and the study has the merits of large sample size and multicenter collaboration.

CONCLUSION

Occult CBC can predict recurrent HCD. Elevated cyst fluid bilirubin and ALP levels predicted POBF and recurrent HCD.

ARTICLE HIGHLIGHTS

Research background

Different methods have been used to treat hydatid cyst of the liver but there are no conclusive results. The two most common complications are postoperative biliary fistula and recurrent hydatid cyst disease (HCD).

Research motivation

We were motivated to conduct this study to evaluate the incidence of occult cysto-biliary communication (CBC) and HCD recurrence after laparoscopic partial resection and omentoplasty.

Research objectives

The objectives of the study were to determine the incidence of occult CBC and HCD recurrence rate with the aim of reducing these two complications. The second objective was to detect an association between high cystic fluid biochemical indices and the incidence of developing postoperative biliary fistula and HCD recurrence.

Research methods

A prospective observational study was conducted involving 244 patients with stage cystic echinococcosis 2 and cystic echinococcosis 3b according to the World Health Organization's Informal Working Group on Echinococcosis classification who underwent laparoscopic partial cystectomy with omentoplasty.

Research results

There was a highly statistically significant association between cystic fluid biochemical indices and the development of biliary complications. There was a highly statistically significant association between biliary complications, biochemical indices, and the occurrence of recurrent HCD.

Research conclusions

This study is the first to propose that occult CBC can predict recurrent HCD. Elevated cyst fluid bilirubin and alkaline phosphatase levels predicted POBF and recurrent HCD. These findings will encourage surgeons to detect occult CBCs to avoid morbidity and mortality due to POBF and recurrent HCD.

Research perspectives

Future studies should focus on the detection of CBCs to decrease the incidence and morbidity of POBF and HCD recurrence.

FOOTNOTES

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Country/Territory of origin: Egypt

ORCID number: Tamer A A M Habeeb 0000-0002-7049-4267; Mauro Podda 0000-0001-9941-0883; Boris Tadic 0000-0001-5400-1015; Vishal G Shelat 0000-0003-3988-8142; Abd-Elfattah Kalmoush 0000-0001-6927-223X; Tarek Zaghloul Mohamed 0000-0002-1523-6214; Hamdi Elbelkasi 0000-0002-9556-6538; Amr Ibrahim 0000-0002-0573-4113; Tamer Mohamed Elshahidy 0000-0002-0089-5727; Mohamed Ibrahim Mansour 0000-0003-4860-4906.

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ORIGINAL ARTICLE

Prospective Study

Role of endoscopic ultrasound and endoscopic ultrasound-guided tissue acquisition in diagnosing hepatic focal lesions

Hussein Hassan Okasha, Hanane Delsa, Abdelmoneim Alsawaf, Ahmed Morad Hashim, Hani M Khattab, Dalia Abdelfatah, Abeer Abdellatef, Amr Albitar

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Hussein Hassan Okasha, Ahmed Morad Hashim, Abeer Abdellatef, Amr Albitar, Department of Internal Medicine, Division of Gastroenterology and Hepatology, Kasr Al-Aini School of Medicine, Cairo University, Cairo 11451, Egypt

Hanane Delsa, Department of Gastroenterology and Hepatology, Cheikh Khalifa International University Hospital, Mohammed VI University of Sciences and Health, Casablanca 82403, Morocco

Hanane Delsa, Research Unit, Mohammed VI Center for Research and Innovation, Rabat 10100, Morocco

Abdelmoneim Alsawaf, Department of Gastroenterology, Barnsley NHS Foundation Trust, Barnsley S75 2EP, United Kingdom

Hani M Khattab, Department of Pathology, Kasr Al-Aini School of Medicine, Cairo University, Cairo 11451, Egypt

Dalia Abdelfatah, Department of Cancer Epidemiology and Biostatistics, National Cancer Institute, Cairo University, Cairo 11451, Egypt

Corresponding author: Hussein Hassan Okasha, MD, Professor, Department of Internal Medicine, Division of Gastroenterology and Hepatology, Kasr Al-Aini School of Medicine, Cairo University, Kasr Al-Aini Street, Cairo 11451, Egypt. okasha.hussein@gmail.com

Abstract

BACKGROUND

Endoscopic ultrasonography (EUS) has become an established method in diagnostic and therapeutic procedures in gastroenterology; however, it has recently gained a growing role in hepatology.

AIM

To evaluate the role of EUS features, strain elastography (SE), and EUS-tissue acquisition in diagnosing hepatic focal lesions (HFLs) that could affect further management.

METHODS

This cross-sectional study included 215 patients with pancreatic, biliary, or

gastrointestinal malignancies referred for EUS examination. HFLs were identified in 43 patients (20%), and EUS-guided tissue acquisition was performed from these lesions.

RESULTS

EUS features were highly sensitive (100%) but much less specific (57%) in diagnosing HFLs; the overall accuracy was 94%. Real-time elastography was also very sensitive (97%) but less specific (67%) in diagnosing HFLs; however, the overall accuracy was 92%. EUS tissue acquisition was extremely sensitive (100%) and specific (100%), with a 100% overall diagnostic accuracy.

CONCLUSION

The diagnostic utility of EUS-guided tissue acquisition was extremely accurate in diagnosing HFLs. EUS characteristics and real-time SE accurately predicted the histological diagnosis of both benign and malignant HFLs.

Key Words: Endoscopic ultrasonography; Hepatic focal lesions; Fine needle aspiration; Fine needle biopsy; Elastography

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Core Tip: This cross-sectional study included 43 patients with hepatic focal lesions among 215 pancreatic, biliary, or gastrointestinal malignant lesions referred for Endoscopic ultrasonography (EUS) examination. EUS tissue acquisition was highly sensitive (100%) and specific (100%), with an overall diagnostic accuracy of 100%.

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INTRODUCTION

Endoscopic ultrasonography (EUS) is one of the main tools used to evaluate the upper and distal parts of the lower gastrointestinal tract and to define pancreatic and hepatobiliary features. The utility of EUS in diagnosing and managing hepatic focal lesions (HFLs) has gained special concern nowadays due to the proximity of the scope to the liver, and its excellent spatial resolution enables real-time images and guided intervention[1-4].

Liver diseases are among the most common worldwide and manifest as diffuse liver diseases and focal hepatic lesions, ranging from benign to malignant. To make a definitive diagnosis of liver diseases, biochemical and imaging investigations and, in some instances, liver biopsies are typically used[2]. The application of EUS in diagnosing liver diseases is a promising technique and should be considered a first-line therapeutic option in selected cases[4].

EUS-guided diagnosis of focal liver lesions by endosonographic features and cytological and histopathological examination of biopsies obtained *via* fine needle aspiration/biopsy (EUS-FNA/FNB) has been shown to significantly improve the diagnosis of solid liver lesions compared to traditional imaging tools[1].

US and computed tomography (CT)-guided FNA/FNB of focal liver lesions are safe and provide high diagnostic accuracy; however, it is sometimes challenging to access subdiaphragmatic and posteriorly located lesions.

Compared to percutaneous and transjugular routes, EUS-FNA/FNB may have better accessibility and diagnostic yield and may be superior for a targeted approach to focal lesions. It provides higher-quality images and allows for more patient comfort[3]. However, there is limited data regarding the accuracy of EUS-guided biopsy of HFLs[5].

Elastography is a US-based imaging modality that provides information about tissue stiffness and can be considered a virtual biopsy. Several elastographic approaches have been developed, such as transient elastography, strain elastography (SE), histograms, and shear wave imaging, which include point shear wave elastography and 2D shear wave elastography[6].

This study evaluated the diagnostic utility of EUS sonographic features, SE, and EUS-FNA/FNB in differentiating benign from malignant liver lesions, including primary and metastatic lesions that may affect further management.

MATERIALS AND METHODS

This cross-sectional study included 215 Egyptian patients who were referred to the internal medicine department, Kasr Al-Aini hospitals, Cairo University for EUS and EUS-FNA/FNB for an assessment of pancreatic or gastrointestinal tumors without or with HFLs detected by contrast abdominal CT or magnetic resonance imaging (MRI). After the EUS examination, 43 out of 215 (20%) patients had HFLs for which EUS-FNA/FNB was performed. All the required data is

collected from the hospital's medical record after ethical approval is obtained from our hospital's ethical committee.

Exclusion criteria include patients unfit for deep sedation and patients with bleeding disorders contraindicating EUS-FNA/FNB.

All patients were subjected to a thorough history taking, clinical examination, abdominal US, CT, or MRI abdomen, and routine laboratory investigations such as complete blood count, hepatic and renal function tests, and coagulation profile, in addition to virological tests for human immunodeficiency virus, hepatitis C virus, and hepatitis B virus and tumor markers including CA-19-9 and alpha-fetoproteins.

EUS and EUS-FNA/FNB were performed by a single endoscopist. It was conducted under deep sedation using a Pentax linear array echoendoscope type EG-3870UTK attached to a Hitachi ultrasound AVIUS machine. A detailed description of the primary tumor and the HFLs regarding their site, size, shape, and number was applied. Based on EUS features, we considered the mass as malignant if any one of the following criteria is present: (1) The presence of peripheral hypoechoic halo; and (2) The presence of mass effect as compression or interruption of the course of a blood vessel or a biliary radicle, or the presence of contour bulge.

Real-time SE scoring was done to all HFLs. We considered grades 1 and 2 as benign and grades 3 and 4 as malignant lesions. EUS-FNA was conducted with 22G Echotip needles from Cook Company; however, the FNB was conducted with 22G Acquire needles from Boston Scientific Company. All biopsies were done by high pressure technique and fanning, at least two passes, no Rapid On-Site evaluation (ROSE) was available in any of the cases. We have targeted the nearest mass to the Echoendoscope, with no intervening vessels along the needle tract and with the presence of a rim of normal liver tissue between the liver capsule and targeted mass to minimize bleeding. The material was spread over a glass slide and fixed by 95% alcohol, whereas formalin blocks were prepared and sent to a single experienced cytologist.

The gold standard of malignant lesions is the FNB as it has extremely high specificity, about 95%-100% in most articles in the literature. All benign-looking lesions were followed up with the disappearance of all cholangitis abscesses under antibiotic therapy, while the two benign liver nodules were constant in size over 6 mo.

The collected data from the 43 patients with HFLs was organized and statistically analyzed using appropriate methods.

RESULTS

The average age of the patients examined was 56, the majority were male (74.42%), and the most common co-morbidities were diabetes mellitus (10 patients; 23.25%), systemic hypertension (8 patients; 18.6%), and ischemic heart disease (6 patients; 13.95%). Most of the HFLs were present in the left lobe of the liver (67.44%).

The cytopathological confirmed malignant lesions were found in 35 (81.4%) of patients, while benign lesions were found in 8 (18.6%) of patients (Table 1). The eight benign lesions were six cholangitis abscesses and two benign liver lesions, likely areas of focal fat depletion. All benign lesions were followed up with the disappearance of all cholangitic abscesses under antibiotic therapy, while the two benign liver nodules were constant in size over 6 mo. The other 35 malignant lesions were five primary hepatocellular carcinomas, one neuroendocrine tumor, and 29 metastatic liver lesions (Figure 1) from malignant pancreatic masses, as proved by cytopathological and histopathological examination after EUS-FNA/FNB (Figure 2A). The mean size was 23.47 mm × 39.19 mm, with an average number of needles passing 1.49 (0.51).

EUS-FNA/FNB was accurate in diagnosing HFLs with 100% sensitivity, specificity, and diagnostic accuracy.

EUS features provisionally diagnosed 38 patients (88.37%) with malignant lesions and five patients (11.63%) with benign ones (Table 2). Thus, 5 out of 8 benign lesions could be correctly diagnosed based on EUS features. On the other hand, 38 cases were reported with malignant lesions, while the actual number evident by histopathology was only 35; thus, three benign cases were incorrectly diagnosed as malignant lesions (Table 3).

Based on Real-Time elastography scoring, 5 patients were suggested to have benign lesions while 38 lesions were suggested to have malignant lesions. Real-time elastography (Figures 2B and 3) was very sensitive (97%) but less specific (67%) in the diagnosis of HFLs; however, the overall accuracy was 92% (Tables 4 and 5).

EUS-FNA needles were used in 20 patients while EUS-FNB were used in 23 patients. No complications were reported in the study. EUS-FNA was done in 20 patients while EUS-FNB was done in 23 patients.

DISCUSSION

Percutaneous CT or US-guided biopsy is the classical diagnostic method for liver masses. However, it has shown some difficulties in diagnosing small liver lesions less than 2 cm, with common complications and many contraindications limiting its use[7,8].

EUS has been broadly used for identifying and managing GIT and pancreaticobiliary diseases[3]. It has become an excellent tool to confirm the pathological diagnosis in combination with EUS-FNA/FNB.

Compared to percutaneous liver biopsy (PC-LB), EUS-guided liver biopsy (ELB) is a new approach to liver parenchyma sampling that has shown promise for safety, patient comfort, and good tissue yield[9]. ELB also allows sampling from multiple sites in the liver, both in the right and left lobes[9,10].

Few studies have verified the efficacy and safety of ELB; however, the review of Sbeit reported a variable diagnostic yield for EUS-guided liver biopsy in focal liver lesions (91%-100%)[4].

Table 1 Cytopathological diagnosis of hepatic focal lesions

Variable	Total, *n* = 43	
	n	%
Cytopathological diagnosis		
Benign	8	18.60
Malignant	35	81.40
Inflammatory	8	18.60
Cholangitic abscess	6	13.94
Cirrhotic nodule	2	4.65
Primary	6	13.95
Hepatocellular carcinoma	5	11.63
Neuroendocrine tumor	1	2.32
Secondary	29	67.44

Table 2 Endoscopic ultrasonography finding of patients with hepatic focal lesions

Variable	Total, *n* = 43
No. of passes	1.49 (0.51)
Shortest diameter size in mm	19 ± 12.8/(3-67)
Longest diameter size in mm	26 ± 19.1/(4-109)
Diagnosis	
Benign	5 (11.63)
Malignant	38 (88.37)

Data are presented as *n* (%) or mean ± SD/(range).

Table 3 Comparison between endoscopic ultrasonography diagnosis and histopathology results

Variable	EUS, *n* = 43		Histopathology, *n* = 43	
	n	%	*n*	%
Benign	5	11.63	8	18.60
Malignant	38	88.37	35	81.40

EUS: Endoscopic ultrasonography.

Table 4 Diagnostic utility of elastography in predicting benign and malignant hepatic focal lesions

Elastography, *n* = 43	*n* (%)
Benign	
Grade 1	0
Grade 2	5 (11.6)
Malignant	38 (88.4)
Grade 3	10 (23.3)
Grade 4	28 (65.1)

Table 5 Comparison between different endoscopic ultrasonography tools regarding their utility in diagnosis of hepatic focal lesions

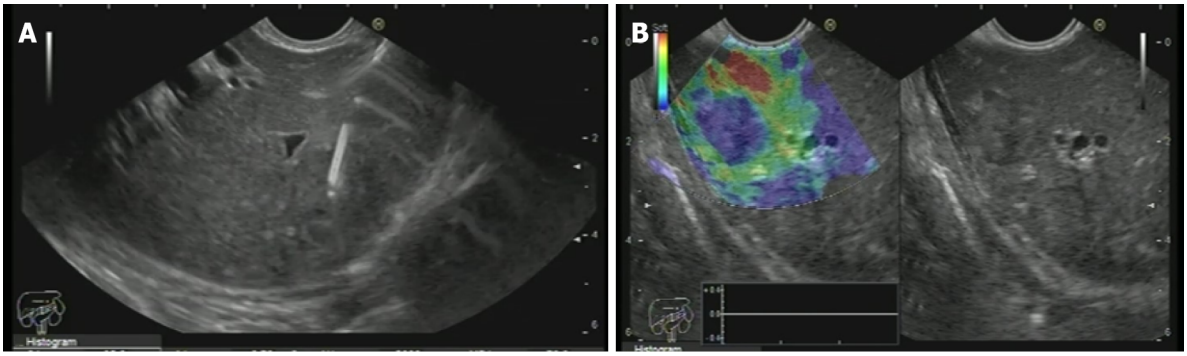
Tool	Sensitivity	Specificity	PPV	NPV	Overall accuracy
Elastography	97	67	94	80	92
EUS	100	57	94	100	94
FNA/FNB	100	100	100	100	100

EUS: Endoscopic ultrasonography; FNA/FNB: Fine needle aspiration/biopsy.



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Figure 1 Multiple hepatic focal lesions due to liver metastasis.

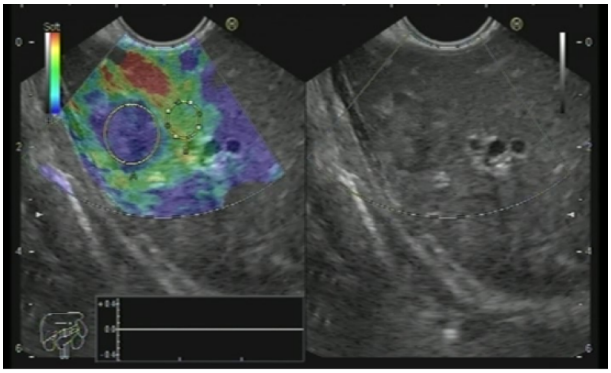


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Figure 2 A metastatic focal hepatic mass. A: Endoscopic ultrasound guided fine needle biopsy from hepatic focal lesion; B: A metastatic focal hepatic mass with grade 4 Elasticity score.

This study aimed to evaluate EUS and EUS-FNA/FNB's diagnostic efficacy in diagnosing liver lesions, whether benign or malignant, including primary and metastatic lesions.

In this study, EUS detected and sampled all HFLs, whereas 88.37% of patients had malignant lesions (liver metastasis) and 11.63% had benign ones. The mean elastographic strain ratio of the HFLs was 3.6 (0.7), and the mean size was 23.47 mm × 39.19 mm, with an average number of needles passing 1.49 (0.51). Finally, considering the cytopathological diagnosis of the biopsy, it proved malignant lesions in 81.4 percent of patients, while benign lesions were found in 18.6%. The eight benign lesions were six cholangitis abscesses and two benign-looking liver lesions. All benign-looking lesions were followed up with the disappearance of all cholangitis abscesses under antibiotic therapy, while the two benign liver nodules were constant in size over 6 mo. The other 35 malignant lesions were five primary hepatocellular carcinomas, one neuroendocrine tumor, and 29 liver metastatic lesions. This finding was consistent with Oh *et al*[11], who investigated the role of EUS-FNA in targeting right-sided liver masses and found that 39 (80.9%) of 47 patients were proven to have malignant lesions. The mean tumor size was 26 mm. The median number of needle passes was 3. On microscopic examination, tissue specimens obtained by EUS-FNA were determined to be adequate in 42 of 46 patients (91.3%). The pathological diagnosis was malignancy in 23 of 46 patients (50%), suspicious for malignancy in 6 patients (13%), atypical in 4 patients (8.7%), and negative for malignancy in 9 patients (19.6%).



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Figure 3 A metastatic focal hepatic mass with high Strain ratio.

Another study by Chon *et al*[12] discussed the role of ELB in diagnosing solid liver lesions. The study included 58 patients (35 males and 23 females) with a mean age (68.0 ± 10.6 years). The mean size of the mass was 21.4 ± 9.16 mm × 11.5 ± 8.15 mm. The biopsy target site was the left lobe in 39 patients, the right lobe in 16 patients, and the caudate lobe in 3 patients. The number of trans-gastric and trans-duodenal route procedures was 39 (67.2%) and 19 (32.8%), respectively. The mean needle pass number was 2.6 ± 0.8, ranging from 1-5 per lesion. The final diagnosis was performed in 52 cases out of 58; all were malignant, either HCC or metastatic.

Adler *et al*[13] performed a multicenter retrospective review of 200 patients, specifically looking at safety and performance when sampling solid lesions. They reported excellent diagnostic yield at 98.5%; however, 6.5% of the patients needed a repeat procedure at some point. No adverse events were identified in the population.

In our study, EUS features were highly sensitive (100%) and less specific (57%) in diagnosing HFL, with an overall accuracy of 94%. Real-time elastography was also highly sensitive (97%) and less specific (67%) for diagnosing HFL; overall accuracy was 92%. EUS tissue acquisition was extremely sensitive (100%) and specific (100%), with an overall diagnostic accuracy of 100%.

Similarly, in 2019, Chon *et al*[12] stated that the diagnostic accuracy for EUS-FNB was 89.7%, but both specimen adequacy for histology, and available immunohistochemistry stain were 91.4%. The sensitivity and specificity of EUS-FNB were 89.7% and 100%, respectively[12].

Two recent meta-analyses reported that ELB and PC-LB are comparable in terms of safety and diagnostic performance [14,15]; however, ELB was more cost-effective than PC-LB regarding lower costs per patient and higher quality-adjusted life years[15].

EUS has the advantage of sampling and evaluating both lobes of the liver and small liver lesions that may have been missed by other non-invasive imaging modalities[16]. This accurately depicts liver histology and potentially addresses concerns about sampling error[17].

A further advantage of ELB over PC-LB was that it permitted more straightforward access to the right and left regions of the liver, thereby reducing the variability in diagnosis. Furthermore, ELB provided a much shorter recovery period (around 4 h) than PC-LB (usually a minimum of 10 h)[18].

In the Oh study, there were no statistical differences in the diagnostic accuracy of ELB between right and left lobe sites (25/28, 89.3% *vs* 13/14, 92.9%, $P = 0.86$), and none of the patients experienced procedure-related adverse events[18]. Similarly, no significant adverse events had been encountered in our study. Liver biopsy is very safe as liver is very near to the Echoendoscope. Also, if intrahepatic hematoma occurred, the blood will trickle to one of the portal or hepatic vessels, so the patient will bleed into its own circulation.

More recently, the meta-analysis by Zeng *et al*[15] suggests that the use of Acquire Franseen-tip needles may increase the ability to obtain more diagnostic samples than Sharkcore Fork-tip needles and that the use of FNB needles may be associated with a higher risk of adverse events than FNA needles[15].

Cholongitas *et al*[19] conducted a systematic review and meta-analysis of over 10000 percutaneous liver biopsies and found that an average of 7.5 core biopsy passes (CPT) and a target specimen length (TSL) of 17.7 mm were necessary for adequate pathological evaluation. However, when the biopsy was obtained through the transjugular route, adequacy was defined as 6.5 CPT and a TSL of 12 mm. While there is no established optimal definition of specimen adequacy for endoscopic ultrasound-guided liver biopsy (EUSLB), the American Association for the Study of Liver Diseases (AASLD) recommends a minimum of 11 CPTs as the definition of adequacy, regardless of the sampling route[19-22]. Additionally, the AASLD guideline suggests a TSL greater than 15 mm to define adequacy, with an ideal size of 30 mm. In this study, all routes of tissue sampling achieved at least 11 CPTs and a TSL greater than 15 mm. However, only EUSLB achieved the ideal TSL of 30 mm or more, which is considered optimal[23].

The study conducted by Ching-Companioni *et al*[24] demonstrated that endoscopic ultrasound-guided liver biopsy (EUS-LB) using a novel 19G FNB needle produced longer and less fragmented biopsy specimens compared to the standard 19G FNA needle. Furthermore, there was a reduced occurrence of specimen fragmentation during post-processing, and the yield of CPT was higher. These findings suggest that utilizing a 19G FNB needle represents an advancement over the conventional 19G FNA needle for EUS-LB[24].

In a prospective crossover randomized controlled trial, which is an appropriate model for assessing two types of tools, the researchers reached the conclusion that EUS-FNB is highly effective for solid liver masses. The newly developed antegrade-bevel needle demonstrated comparable efficacy and incidence of adverse events to the original reverse-bevel needle. However, the antegrade-bevel needles were able to obtain a larger amount of biopsy tissue compared to the reverse-bevel needles[25].

In a systematic review and meta-analysis examining the feasibility, safety, and usefulness of EUS-LB in patients undergoing parenchymal liver biopsy, the researchers found that the combined analysis of multiple studies demonstrated a significant diagnostic success rate of over 90%. This rate is similar to the diagnostic yield achieved by traditional PC-LB [26].

In another meta-analysis study, a comprehensive analysis was conducted on published studies examining the effectiveness and safety of EUS-LB for liver parenchymal diseases and focal liver lesions. The study assessed various outcomes including diagnostic yield, specimen adequacy, qualified specimens with the assistance of ROSE, and adverse events. The pooled analysis revealed that EUS-LB proved to be a highly effective and safe technique, with a successful pathological diagnosis rate of 95%, an adequate specimen rate of 84%, and an adverse events rate of 3%. Subgroup analyses were also performed, which indicated that Acquire Franseen-tip needles exhibited a higher diagnostic yield compared to SharkCore Fork-tip needles (99% *vs* 88%, $P = 0.047$). Moreover, FNB needles showed a higher risk of adverse events in comparison to FNA needles (6% *vs* 1%, $P = 0.028$). Interestingly, no significant differences were observed between 19 G and 22 G needles. Additionally, no significant disparities were identified between FNB and FNA needles in relation to our primary outcomes[15].

In order to improve the quality and accuracy of EUS, elastography has been developed, which allows the assessment of liver tissue firmness and the characterization of HFL. Real-time elastography showed high sensitivity (92.5%) and specificity (88.8%) with reasonable accuracy (88.6%) in a study by Sandulescu *et al*[27].

Innovations in needle technology and new approaches using ELB are in development, with the possibility of concomitant procedures such as EUS portal pressure gradient measurement, another emerging area in the field of endohepatology, in the coming years[8].

CONCLUSION

The diagnostic utility of EUS-LB with FNA/FNB was perfect and the best diagnostic tool for the definitive diagnosis of the HFLs. Furthermore, EUS features during the procedures provided an excellent and accurate prediction of the histological diagnosis, determining whether the lesion was benign or malignant.

ARTICLE HIGHLIGHTS

Research background

Endoscopic ultrasonography (EUS) has become an established method in diagnostic and therapeutic procedures in gastroenterology; however, it has recently gained a growing role in hepatology.

Research motivation

EUS tissue acquisition was highly sensitive (100%) and specific (100%), with an overall diagnostic accuracy of 100%.

Research objectives

This study aimed to evaluate the role of EUS features, strain elastography (SE), and EUS-tissue acquisition in diagnosing hepatic focal lesions (HFLs) that could affect further management.

Research methods

This cross-sectional study included 215 patients with pancreatic, biliary, or gastrointestinal malignancies referred for EUS examination. HFLs were identified in 43 patients (20%), and EUS-guided tissue acquisition was performed from these lesions.

Research results

EUS features were highly sensitive (100%) but much less specific (57%) in diagnosing HFLs; the overall accuracy was 94%. Real-time elastography was also very sensitive (97%) but less specific (67%) in diagnosing HFLs; however, the overall accuracy was 92%. EUS tissue acquisition was extremely sensitive (100%) and specific (100%), with 100% overall diagnostic accuracy.

Research conclusions

The diagnostic utility of EUS-guided tissue acquisition was extremely accurate in diagnosing HFLs. EUS characteristics and real-time SE accurately predicted the histological diagnosis of both benign and malignant HFLs.

Research perspectives

This cross-sectional study included 43 patients with HFLs among 215 pancreatic, biliary, or gastrointestinal malignant lesions referred for EUS examination. EUS tissue acquisition was highly sensitive (100%) and specific (100%), with an overall diagnostic accuracy of 100%.

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FOOTNOTES

Author contributions: Okasha HH was the main EUS endoscopist; Delsa H and Alsawaf A collected the data; Abdellatef A revised and submitted the manuscript; Khattab HM was the main pathologist; Abdelfatah D wrote the statistics; Albitar A wrote the manuscript.

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Country/Territory of origin: Egypt

ORCID number: Hussein Hassan Okasha 0000-0002-0815-1394; Hanane Delsa 0000-0001-7008-8552; Abdelmoneim Alsawaf 0000-0002-6583-2858; Ahmed Morad Hashim 0000-0001-9563-7618; Hani M Khattab 0000-0001-5145-6081; Dalia Abdelfatah 0000-0002-8598-7128; Abeer Abdellatef 0000-0001-9945-9767; Amr Albitar 0000-0002-7968-4382.

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SYSTEMATIC REVIEWS

Post-COVID-19 cholangiopathy: Systematic review

Mazen Abdalla Rasheed, Vinícius Remus Ballotin, Lucas Goldmann Bigarella, Jonathan Soldera

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Mazen Abdalla Rasheed, Jonathan Soldera, Acute Medicine, University of South Wales, Cardiff CF37 1DL, United Kingdom

Vinícius Remus Ballotin, Lucas Goldmann Bigarella, School of Medicine, Universidade de Caxias do Sul, Caxias do Sul 95070-560, Brazil

Corresponding author: Jonathan Soldera, MD, MSc, Tutor, Acute Medicine, University of South Wales, 86-88 Adam St, Cardiff CF37 1DL, United Kingdom.
jonathansoldera@gmail.com

Abstract

BACKGROUND

The coronavirus disease 2019 (COVID-19) pandemic has had a profound impact on global health, primarily characterized by severe respiratory illness. However, emerging evidence suggests that COVID-19 can also lead to secondary sclerosing cholangitis (SC), referred to as post-COVID-19 cholangiopathy.

AIM

To synthesize currently reported cases to assess the current state of knowledge on post-COVID-19 cholangiopathy.

METHODS

Medical Subject Headings and Health Sciences Descriptors were used to retrieve relevant studies, which were combined using Boolean operators. Searches were conducted on electronic databases including Scopus, Web of Science, and MEDLINE (PubMed). Studies published in English, Spanish, or Portuguese were included, with no restrictions on the publication date. Additionally, the reference lists of retrieved studies were manually searched. Simple descriptive analyses were used to summarize the results. Then the data were extracted and assessed based on Reference Citation Analysis (https://www.referencecitationanalysis.com/).

RESULTS

The initial search yielded a total of 192 articles. After screening, 85 articles were excluded due to duplication, leaving 107 articles for further review. Of these, 63 full-length articles met the inclusion criteria and were included in the analyses. Most of the patients were male and exhibited elevated liver function tests (93.8%). Magnetic resonance imaging revealed duct thickening with contrast enhancement (47.7%), as well as beading of the intrahepatic ducts (45.7%) with peribiliary contrast enhancement on diffusion (28.7%). Liver biopsy results confirmed SC in most cases (74.4%). Sixteen patients underwent liver transplantation, with three

experiencing successful outcomes.

CONCLUSION

Post-COVID-19 cholangiopathy is a serious condition that is expected to become increasingly concerning in the coming years, particularly considering long COVID syndromes. Although liver transplantation has been proposed as a potential treatment option, more research is necessary to establish its efficacy and explore other potential treatments.

Key Words: Coronavirus disease 2019; Severe acute respiratory syndrome coronavirus 2; Cholangiopathy; Liver function tests; Liver transplantation

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Core Tip: Post-coronavirus disease 2019 (COVID-19) cholangiopathy is a rare but serious complication that can occur after contracting COVID-19. It is characterized by inflammation and damage to the bile ducts. To better understand this condition and its treatment, we conducted a systematic review of post-COVID-19 cholangiopathy cases. Sixty-three articles met the inclusion criteria, representing 540 patients. Males over 50-years-old were more prone to this condition, which is often accompanied by elevated liver function, bile duct thickening, and kidney failure after prolonged use of mechanical ventilation. Further research is needed to confirm the effectiveness of liver transplantation in treating post-COVID-19 cholangiopathy.

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INTRODUCTION

On March 2020, the World Health Organization declared a global health pandemic after the first case was recognized on December 2019 in Wuhan City, China, of what was called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. This led to catastrophic events in the world resulting in more than 6 million deaths globally. The pandemic has led to a great financial and humanitarian loss due to prolonged lockdowns, which have had a tragic effect on the global economy[2].

Also, coronavirus disease 2019 (COVID-19) keeps enduring second and third waves of outbreaks in many countries, probably caused by mutant new variants of the virus[2]. Despite the accelerated speed of vaccine development for the prevention of COVID-19 to control the disease and robust mass vaccination worldwide including booster doses, these new SARS-CoV-2 variants threaten the progress made so far with the purpose of controlling the spread of the disease[2, 3].

Respiratory symptoms are the most common manifestation of the disease, which range from mild to severe and may include fever, dry cough, shortness of breath, anosmia, ageusia, and fatigue[4]. It may lead to viral pneumonia with severe complications such as acute respiratory failure, acute respiratory distress syndrome requiring intubation, mechanical ventilation (MV), and intensive care management[5,6].

In addition to respiratory symptoms, COVID-19 might also cause a range of extrapulmonary manifestations including cardiovascular, neurological, and renal complications[7]. Gastrointestinal symptoms, including diarrhea, nausea, and vomiting, are also commonly reported[8]. Post-COVID-19, derangement of liver enzymes is a potential complication observed in admitted COVID-19 patients, with a prevalence ranging from 14% to 83%[9]. Other liver-related conditions such as autoimmune hepatitis, vascular thrombosis, and hemophagocytic lymphohistiocytosis have also been associated with the post-COVID-19 period[9,10].

However, one emerging complication of COVID-19 is post-COVID-19 cholangiopathy (PCC), a novel clinical entity characterized by inflammation and damage to the bile ducts in individuals who have recovered from COVID-19 infection [11]. The clinical presentation of PCC can vary, but common symptoms may include abdominal pain, fever, and jaundice [12]. PCC has been observed in patients without a history of prior liver disease. This condition can manifest in various clinical settings, such as in individuals with severe COVID-19 infection requiring MV, as well as in those experiencing milder forms of the disease[5,13]. The prevalence of PCC is not well understood, and it is not clear if it is more common in certain patient populations. Some researchers have suggested a potential association between certain drugs, including immunomodulator agents, ketamine, and antiviral medications, and the development of PCC. However, the available evidence regarding these drugs causing cholangiopathy remains insufficient[9].

This systematic review comprehensively analyzes and synthesizes the existing evidence pertaining to PCC. The primary objective is to explore the clinical presentation and management approaches documented in the available cases reported in the literature. By conducting this review, we provide a comprehensive overview of the current understanding

and knowledge gaps surrounding PCC, which can contribute to the development of effective strategies for diagnosis and treatment in clinical practice.

MATERIALS AND METHODS

Study design

This study was conducted in accordance with the guidelines for preferred reporting items for systematic reviews and meta-analyses (PRISMA) protocol guidelines[14].

Data sources

The studies included in this review were identified using the search strategy: in ("COVID-19" OR "SARS-COV-2") AND ("cholangiopathy" OR "cholangitis" OR "liver transplantation"). This search command was run on the electronic databases Scopus, Web of Science, and Medline (PubMed). Languages were restricted to English, Spanish, and Portuguese. There was no date of publication restrictions. The reference lists of the retrieved studies were also manually searched. The databases were searched in March 2023. Reference Citation Analysis (https://www.referencecitationanalysis.com/) was used to supplement the search.

Inclusion and exclusion criteria

Inclusion criteria were clinical case reports or case series of post-COVID cholangiopathy. Studies needed to include detailed information about the clinical presentation, diagnosis, management, and outcomes. Articles unrelated to the topic were excluded as were those that did not provide sufficient detail about the cases. If there was more than one study published using the same case, the variables were complemented with both articles. Studies published only as abstracts were included, as long as the available data made data collection possible.

Study selection and data extraction

A comprehensive search of various databases was conducted using the search terms listed in the COVID-19, cholangitis, and liver transplantation ("COVID-19" OR "SARS-COV-2") AND ("cholangiopathy" OR "cholangitis" OR "liver transplantation"). The initial screening process involved reviewing titles and abstracts to identify potentially relevant studies. These studies were then analyzed in full, and some were excluded due to a lack of clinical information. Two reviewers independently extracted data from eligible studies using a standardized form and assessed the characteristics of the subjects and outcomes measured. Any discrepancies in study selection or data extraction were resolved by a third party.

Data collection

Variables included were age, sex, clinical presentation, liver function tests, renal function test, imaging findings, histopathology, whether or not the patient had undergone orthotopic liver transplantation (OLT), and outcome.

Data processing and analysis

Data were analyzed and summarized using descriptive techniques such as frequency, means, and median. The analyses were performed using Microsoft Excel 2010.

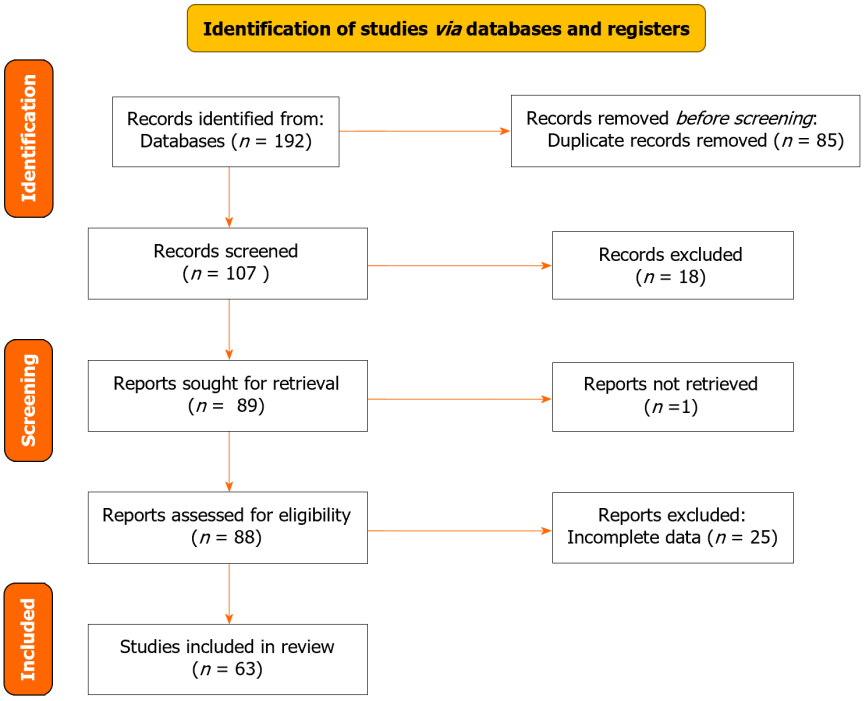
RESULTS

The search strategy retrieved 192 articles; 85 articles were excluded because they were duplicates and 107 articles were screened in the review. A total of 88 full-length articles were included and retrieved, of which 63 were included in the review. The PRISMA flowchart illustrating the search strategy is shown in Figure 1. Studies reviewed were either a case report or a case series.

This systematic review included a total of 540 patients, of whom 69 (12.7%) were male, 26 (4.8%) were female, and 445 (82.5%) did not note their sex. The majority of patients (66, 12.2%) were over 50-years-old. Almost all patients (93.8%) had elevated liver enzymes in the acute phase, with an increase of these levels in the chronic phase. Total bilirubin was elevated in 343 patients (63.5%), while only 80 (14.8%) had levels lower than 1.2 mg/dL. Data on bilirubin levels were not reported for 19 cases. Levels of alkaline phosphatase were high among 488 patients (90.3%) and gamma-glutamyl transferase were consistently elevated, often surpassing 1000 U/L.

In this study, based on imaging findings, 225 of 540 (41.6%) patients had biliary ductal dilatation with fibrosis on ultrasound, while 50 (9.2%) patients did not show any alteration. Furthermore, according to magnetic resonance imaging (MRI) results, 258 (47.7%) patients had bile duct thickening with contrast enhancement, 247 (45.7%) had beading of the intrahepatic ducts, and 155 (28.7%) had peribiliary enhancement on diffusion.

Moreover, 223 (41.3%) patients with PCC had respiratory failure type 2, which was characterized by acute respiratory distress syndrome (ARDS). Some of these patients underwent bilateral lung transplantation, but unfortunately 1 patient died. Additionally, 355 patients (65.7%) had acute renal injury that required either dialysis or renal transplantation after OLT. Data on renal function were not reported for 16 patients. According to liver biopsy results, 402 patients (74.4%) had



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Figure 1 The preferred reporting items for systematic reviews and meta-analyses flowchart for the systematic review.

sclerosing cholangitis (SC). Moreover, 16 patients (2.96%) with post-COVID-19 cholangitis underwent OLT. Of these, 15 patients experienced successful outcomes, with an improvement in liver enzyme levels post-transplantation.

DISCUSSION

After the first of case of SARS-CoV-2 disease in 2019[1], a novel clinical entity emerged. This condition has been reported in a small number of patients who have recovered from the virus and is characterized by elevated liver enzymes, biliary ductal dilatation on imaging, and histopathological findings of secondary SC (SSC)[11]. This systematic review examined the clinical presentations and outcomes of 540 patients with PCC, a rare complication of COVID-19 that affects the biliary system.

It is important to consider the differential diagnosis, as other diseases may present with a similar presentation[15]. Ketamine-induced cholangiopathy can lead to fusiform dilatation of the common bile ducts, without evidence of extrinsic or intrinsic obstruction[16]. The severity depends on the duration of using ketamine, and it is reversible in abstinent patients. Another difference is ischemic cholangitis, which occurs as a consequence of deficient blood flow to the bile duct wall[17]. This can affect the bile ducts leading to segmental strictures and cholangiectasis, resulting in mechanical restriction of bile acid flow.

SC is a medical condition characterized by the destruction of bile ducts due to inflammation and fibrosis and severe progressive stenosis of the bile tracts including three types: primary SC (PSC); immunoglobulin G-related SC (IgG-SC); and secondary cholangitis such as bacterial cholangitis, viral cholangitis (cytomegalovirus), postoperative biliary stenosis, and choledocholithiasis. Usually the patients present with similar cholestatic features such as itching and jaundice, and blood tests reveal high cholestatic enzymes[18,19]. Although the clinical presentation of PSC and IgG4-SC are nearly the same, they differ in treatment response, outcomes and comorbidities, and how to differentiate it from cholangiocarcinoma[18,19]. The difference between them is that IgG4-SC patients respond actively to prednisolone and steroid therapy, whereas PSC has no standard treatment approved; only ursodeoxycholic acid can be used in some patients, but it does not improve the overall prognosis[18-21].

Distinguishing and differentiating between PSC-high IgG and IgG-SC is challenging. A promising study that calculated serum IgG4:IgG1 ratios showed excellent specificity in distinguishing IgG4-SC from PSC-high IgG4[18,19]. The most common diagnostic test for PSC is cholangiography, which shows a pruned tree appearance, beaded ducts, and band-like stricture; thus, endoscopic retrograde cholangiopancreatography (ERCP) or magnetic resonance cholangiopancreatography is highly recommended. PSC is also highly associated with inflammatory bowel disease (ulcerative colitis more than Crohn's disease); thus, a colonoscopy is recommended for the diagnosis, which increases the risk of cholangiocarcinoma and gallbladder carcinoma[18,19]. Therefore, more studies are required on the diagnostic procedures of PSC, IgG4-SC, and cholangiocarcinoma and their treatment and management[18,19].

The present results on PCC showed that most patients are male (12.7%) older than 50-years-old, consistent with the previous literature[9]. Every patient had elevated liver enzymes in the acute phase, and their levels increased in chronic

Table 1 Baseline features in 540 patients with post-COVID-19 cholangiopathy, *n* (%)

Variable	Patients, *n* = 540 (100)
Sex	
Male	69 (12.7)
Female	26 (4.8)
Age > 50 year	66 (12.2)
Liver enzymes	
High (> 45)	507 (93.8)
Total bilirubin	
High (> 1.2 mg/dL)	343 (63.5)
Alkaline phosphatase	
High (> 147 IU/L)	488 (90.3)
Ultrasound findings	
Biliary ductal dilatation with fibrosis	225 (41.6)
MRI Findings	
Bile duct thickening and enhancement	258 (47.7)
Beading of intrahepatic ducts	247 (45.7)
Peribiliary diffusion	155 (28.7)
Histopathology with secondary sclerosing cholangitis	402 (74.4)
Orthotopic liver transplantation	16 (2.96)

COVID-19: Coronavirus disease 2019; MRI: Magnetic resonance imaging.

phase if left untreated.

Also, ultrasound findings showed that 225 patients (41.6%) presented with biliary ductal dilatation. The MRI findings in this systematic review showed that only a small number of patients (28.7%) had peribiliary enhancement on diffusion, while a larger number of patients (47.7%) had bile duct thickening and enhancement, and 247 patients (45.7%) had beading of the intrahepatic ducts. By contrast, a previous retrospective study by Faruqui *et al*[13] showed that a higher proportion of patients (11/12, 92%) had beading of the intrahepatic ducts, 7/12 (58%) had bile duct wall thickening with enhancement, and 10/12 (83%) had peribiliary diffusion high signal[11]. Details can be found in Table 1 and Table 2.

PCC appears to have different histologic characteristics compared to SSC in critically ill patients caused by other factors. Biopsy samples from patients with PCC show extensive degeneration and injury of cholangiocytes, as well as unique microvascular features such as swelling of hepatic artery endothelial cells, phlebitis in the portal vein, and sinusoidal obstruction syndrome[5]. Several studies have suggested that COVID-19 cholangiopathy is the result of progressive paucity of bile ducts; however, the exact pathophysiology is not well known[11]. Our histopathology biopsy results showed SSC in 402 patients (74.4%).

On the other hand, PCC presentation is difficult to treat, and sometimes requires OLT[5,6,21]. Almost all patients presented with respiratory failure type 2 as they had ARDS, and 1 patient had bilateral lung transplant and unfortunately died. Every patient presented with acute kidney injury, which required either dialysis or renal transplantation post OLT. As described in the literature, PCC is often accompanied by respiratory failure and acute renal injury[22-25]. Also, some cases of biliary casts have been described, removed *via* ERCP. The diagnosis and management of post-COVID 19 cholangiopathy requires an ERCP, especially in the presence of a dilated choledocus in imaging studies[9,26].

Also, 16 patients (2.96%) underwent OLT, which can be a viable treatment option for this condition[5,27]. One of these cases was reported by Durazo *et al*[5], which comprised SSC in a 47-year-old patient who was recovering from severe acute respiratory distress syndrome caused by COVID-19 infection. He was admitted to the intensive care unit for prolonged MV (29 d) and was listed for liver transplantation with a model for end-stage hepatic disease score of 37. On day 108 from his presentation, the patient underwent successful OLT with a whole liver allograft from a deceased donor.

CONCLUSION

In conclusion, this paper presents an extensive review of post-COVID-19 cholangiopathy published in medical journals. Our analysis indicates that post-COVID-19 cholangiopathy is a serious systemic illness that can affect the liver in addition to the lungs. Most cases were found in males over 50-years-old, and patients with cholangiopathy exhibited elevated liver

Table 2 Summary of systemically reviewed clinical cases

Ref.	Age, yr	Sex	Clinical presentation	Elevated liver enzyme	U/S findings	MRI findings	Respiratory failure	Renal failure	Histopathology	OLT	Outcome
Roth *et al* [28], 2021	38	Male	Post-COVID-19 cholangiopathy	Yes	Intrahepatic bile ducts beading, with sub-segmental strictures and dilatation	Beading of intrahepatic ducts	Yes, required MV; On supplemental oxygen, then off on day 63 and decannulated	Yes, recovered	Portal tract findings; Mild duct paucity, moderate bile duct swelling & reaction; Mild portal tract inflammation; Endothelial hepatic artery swelling; Portal veins with focal endo phlebitis	Not done	Recovered
	25	Male	Post-COVID-19 cholangiopathy	Yes	Hepatomegaly, extrahepatic bile duct dilatation, intrahepatic bile duct dilatation	Beading of intrahepatic ducts	Yes, required MV; On supplemental oxygen, then off on day 112 and decannulated	Yes, recovered	Portal tract findings; Moderate duct paucity, moderate bile duct swelling & reaction. Moderate portal tract inflammation; Endothelial hepatic artery swelling; Portal veins with focal endo phlebitis	Not done	Recovered
	40	Female	Post-COVID-19 cholangiopathy	Yes	Hepatomegaly, no dilatation	Peribiliary diffusion, moderate portal and periportal fibrosis	Yes, remains with tracheostomy & MV, and then off MV on day 63	Yes, recovered	Portal tract findings; Moderate duct paucity, moderate bile duct swelling & reaction; Severe portal tract inflammation; Endothelial hepatic artery swelling; Portal veins with focal endo phlebitis	Not done	Death, cardiac arrest
Faruqui *et al* [13], 2021	Mean age 58	Male	Post-COVID-19 cholangiopathy	Yes	U/S showed; extrahepatic bile duct dilatation and intrahepatic bile duct dilatation and periportal diffusion	MRI showed, beading of intrahepatic ducts (11/12, 92%); Peribiliary diffusion (10/12, 83%); Bile duct wall thickening (7/12, 58%)	Patients required MV	Yes, recovered	Large duct obstruction without clear bile duct loss	Done OLT	Had t successful recovery and rapid clinical improvement
	Mean age 58	Female	Post-COVID-19 cholangiopathy	Yes	Experiencing persistent	Beading of intrahepatic ducts;	Patients required MV	Yes, recovered	Large duct obstruction without	Highly recommended	Recovery with long-term

				jaundice, hepatic insufficiency, and/or recurrent bacterial cholangitis	Peribiliary diffusion; Bile duct wall thickening			clear bile duct loss	for OLT. Patient on transplantation waiting list, still not done OLT at time of study	liability and comorbidity
Mean age 58	Male	Post-COVID-19 cholangiopathy	Yes	Experiencing persistent jaundice, hepatic insufficiency, and/or recurrent bacterial cholangitis	Beading of intrahepatic ducts; Peribiliary diffusion; Bile duct wall thickening	Patients required MV	Yes, recovered	Large duct obstruction without clear bile duct loss	Highly recommended for OLT. Patient on transplantation waiting list, still not done OLT at time of study	Recovery with long-term liability and comorbidity
Mean age 58	Male	Post-COVID-19 cholangiopathy	Yes	Experiencing persistent jaundice, hepatic insufficiency, and/or recurrent bacterial cholangitis	Beading of intrahepatic ducts; Peribiliary diffusion; Bile duct wall thickening	Patients required MV	Yes, recovered	Large duct obstruction without clear bile duct loss	Highly recommended for OLT. Patient on transplantation waiting list, still not done OLT at time of study	Recovery with long-term liability and comorbidity
Mean age 58	Male	Post-COVID-19 cholangiopathy	Yes	Experiencing persistent jaundice, hepatic insufficiency, and/or recurrent bacterial cholangitis	Beading of intrahepatic ducts; Peribiliary diffusion; Bile duct wall thickening	Patients required MV	Yes, recovered	Secondary sclerosing cholangitis	Highly recommended for OLT, patient on transplantation waiting list, still not done OLT at time of study	Recovery with long-term liability and comorbidity
Mean age 58	Male	Post-COVID-19 cholangiopathy	Yes	Intrahepatic bile duct dilatation and periportal diffusion	Beading of intrahepatic ducts; Peribiliary diffusion; Bile duct wall thickening	Patients required MV	Yes, recovered	Secondary sclerosing cholangitis	OLT Not done	Recovery with long-term liability and comorbidity
Mean age 58	Male	Post-COVID-19 cholangiopathy	Yes	Intrahepatic bile duct dilatation and periportal diffusion	Beading of intrahepatic ducts; Peribiliary diffusion; Bile duct wall thickening	Patients required MV	Yes, recovered	Secondary sclerosing cholangitis	OLT Not done	Recovery with long-term liability and comorbidity
Mean age 58	Male	Post-COVID-19 cholangiopathy	Yes	Intrahepatic bile duct dilatation and periportal	Beading of intrahepatic ducts; Peribiliary diffusion	Patients required MV	Yes, recovered	Secondary sclerosing cholangitis	OLT Not done	Recovery with long-term liability and comorbidity

					diffusion						
	Mean age 58	Male	Post-COVID-19 cholangiopathy	Yes	Intrahepatic bile duct dilatation and periportal diffusion	Beading of intrahepatic ducts; Peribiliary diffusion	Patients required MV	Yes, recovered	Secondary sclerosing cholangitis	OLT Not done	Recovery with long-term liability and comorbidity
	Mean age 58	Male	Post-COVID-19 cholangiopathy	Yes	Intrahepatic bile duct dilatation and periportal diffusion	Beading of intrahepatic ducts; Peribiliary diffusion	Patients required MV	Yes, recovered	Secondary sclerosing cholangitis	OLT Not done	Recovery with long-term liability and comorbidity
	Mean age 58	Male	Post-COVID-19 cholangiopathy	Yes	Intrahepatic bile duct dilatation and periportal diffusion	Beading of intrahepatic ducts	Patients required MV	Yes, recovered	Secondary sclerosing cholangitis	OLT Not done	Recovery with long-term liability and comorbidity
	Mean age 58	Male	Post-COVID-19 cholangiopathy	Yes	Intrahepatic bile duct dilatation and periportal diffusion	MRI not available	Patients required MV	Yes, recovered	Secondary sclerosing cholangitis	OLT Not done	Recovery with long-term liability and comorbidity
Li *et al*[29], 2022	N/A	Two sample mendelian randomization	The autoimmune diseases showed not associated with COVID-19 infection	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Hunyady *et al*[30], 2023	N/A	24 Patients	Post-COVID-19 cholangiopathy developed after a median of 91 d	Yes	N/A	N/A	Patients required MV, the median was 48 d among all patients	N/A	COVID-SSC and CIP-SSC share the same clinical phenotype	N/A	UDCA showed great improvement in patients without liver cirrhosis and reduced severity in patients with liver cirrhosis, while OLT showed significant improvement in patient with liver cirrhosis
Weaver *et al*[31], 2021	63	Male	Post-COVID-19 cholangiopathy	Yes	Sludge in the gallbladder, no biliary ductal dilation, and patent vasculature	N/A	Patients required MV	N/A	Filling defects in the common bile duct as well as an irregular and beaded appearance of the intrahepatic ducts	Not done	Recovered, after ERCP sphincterotomy followed by balloon sweep of the biliary ducts and removal of thick stone

Hartl *et al* [32], 2022	N/A	N/A	Post-COVID-19 cholangiopathy (65 patients with CLD of 496 patients included in the study, around 24.6% non-ACLD *vs* ACLD 10.6% associated with COVID	Yes. Alkaline phosphatase showed (pre: 91.0 *vs* T1: 121.0 *vs* last: 175.0 U/L) and gamma glutamyl transferase GGT (pre: 95.0 *vs* T1: 135.0 *vs* last: 202.0 U/L)	N/A	N/A	N/A	N/A	20% of patients with CLD developed progressive cholestasis post-COVID-19 cholangiopathy, and patients with NASH/NAFLD also have a risk of developing cholestatic liver failure and secondary sclerosing cholangitis post-COVID-19	N/A	N/A
Duengelhoef *et al*[33], 2022	N/A	N/A	Post-COVID-19 cholangiopathy, associated more with Autoimmune hepatitis AIH as well as post COVID vaccine than PBC and PSC patients	Yes	N/A	N/A	N/A	N/A	N/A	N/A	N/A
John *et al* [34], 2023	N/A	N/A	Post-COVID -19 cholangiopathy study included 1607 patients with liver cirrhosis used UDCA	Yes	N/A	N/A	N/A	N/A	N/A	N/A	UDCA showed great improvement in patients with liver cirrhosis, by decreased symptoms and decreased COVID-19 infection
Heucke *et al* [35], 2022	N/A	48	Post-COVID-19 cholangiopathy 13% from 496 patients developed CLD; 23% of patients with CLD developed cholestasis/cholangiopathy	Yes (ALT & AST were elevated in 50 patients less than 5 times upper limit of normal. While in late-stage alkaline phosphatase and GGT were highly progressively elevated	N/A	N/A	Yes, require oxygen supply & some patients MV	Yes, some patients required dialysis for renal failure	The histopathology reported SARS-CoV-2 RNA and/or proteins in human liver tissue and bile samples, this SARS-CoV-2 RNA may lead to provoke a strong proinflammatory cytokine response (TNF, IL□1, IL□6) with hypercoagulation, endothelial damage, consecutive venous and arterial	9 patients listed for OLT and 6 patients done OLT with good recovery	16 patients died, and 24 patients were treated with ketamine during the acute phase of COVID-19 and around 28 patients with SSC from 48 were reduced after using UDCA treatment

									embolism, as well as secondary parenchymal damage		
Bazerbachi *et al*[36], 2022	56	Female	Post-COVID-19 cholangiopathy	Yes alkaline phosphatase 1574U/L, total bilirubin 11 mg/dL, ALT 88 U/L, AST 101 U/L	EUS showed a left hepatic duct stricture and heterogenous, non-shadowing cylindric objects in the main bile duct	N/A	Yes, require tracheostomy & MV	Yes, developed renal failure and required hemodialysis	LHD stricture with upstream dilation of the left ducts, and obliteration of right intrahepatic with secondary sclerosing changes	Not done	Improved, casts were swept and removed, and left lobe was stented with a 10 Fr 20 cm plastic stent improving bilirubin level to a baseline of 3 mg/dL
Cho *et al*[37], 2022	47	Female	Post-COVID-19 cholangiopathy	Yes, highly elevated ALP-positive ANA, anti-mitochondrial highly positive	N/A	N/A	N/A	N/A	N/A	N/A	Post-COVID-19 cholangiopathy may be due to direct cytotoxicity from SARS-CoV-2 active replication, hypoxia induced respiratory failure, drug induced liver injury, vascular coagulopathy, immune mediated liver damage
	57	Male	Post-COVID-19 cholangiopathy	Yes, hypogammaglobinemia, high GGT, elevated AST/ALT, positive anti-mitochondrial antibody, anti-smooth muscle antibodies, and anti-double stranded DNA antibodies	N/A	N/A	N/A	N/A	N/A	OLT Considered for some patients	
	N/A	N/A	Post-COVID-19 cholangiopathy	Yes, ALP > three times	N/A	MRCP showed dilatation of hepatic ducts with stenosis and beading of intrahepatic ducts	N/A	N/A	N/A		
	N/A	24 Patients	Post COVID-19 Cholangiopathy	Yes	N/A	N/A	N/A	N/A	N/A		
Yu *et al*[38], 2022	N/A	N/A	Post-COVID-19 cholangiopathy	Yes	N/A	N/A	N/A	N/A	N/A	N/A	The patients are not only related to liver disease, but also cholangitis may be due to viral cholangitis, systemic inflammation response, and hypoxic liver injury
Sanders *et al*	57	Male	Post-COVID-19 cholan-	Yes	Dilated CBD	N/A	Yes, required	Renal	N/A	N/A	Improved,

[39], 2021			giopathy		with a distal CBD stone		MV. (Tracheostomy & gastrostomy)	impairment required fluid resuscitation			biliary cast removed by ERCP, and bile duct stent and patient referred for cholecystectomy
López Romero-Salazar *et al* [40], 2022	76	Male	Post-COVID-19 cholan-giopathy	Yes, elevated ALT & AST developed AIH and complicated to liver cirrhosis secondary to primary biliary cholangitis (PBC) igg positive, ANA	U/S showed hepatic fibrotic inflammation, dilated lobes, and biliary ducts	N/A	N/A	N/A	Biopsy showed lobular hepatitis, with intense interface, centrilobular necrosis with lymphoplasmacytic inflammation	N/A	The patient has poor prognosis due to liver cirrhosis, the study emphasizes the hypothesis that AIH induced due to or post COVID-19 vaccination. Patient given UDCA and obeticholic acid
Wall *et al* [41], 2022	N/A	N/A	Post-COVID-19 cholan-giopathy	Yes	N/A	N/A	N/A	N/A	N/A	N/A	The study showed to avoid using SARS-CoV-2-positive donors for liver transplantation unless there is a justifying indicator such as recipient illness severity
Ghafoor *et al* [42], 2022	Mean Age 60.5	15 Male patients	Post-COVID-19 cholan-giopathy	Yes	N/A	All patients had intrahepatic bile duct strictures and 10 patients had associated upstream dilatation. Fourteen patients showed intrahepatic bile duct beading. One patient had extrahepatic bile duct structuring; 9 patients showed high signal on T2 and diffusion weighted images & 7 patients showed	N/A	N/A	N/A	N/A	The post-COVID-19 cholangiopathy patients showed on MRI/MRCP multiple intrahepatic bile duct strictures with intrahepatic bile duct beading

						patchy arterial phase hyperenhancement; 2 patients showed biliary casts. Vascular complication, and periportal lymphadenopathy were not seen on MRI/MRCP					
Singh *et al* [43], 2021	57	Male	Post-COVID-19 cholangiopathy	Yes, elevated ALT, AST, GGT, hypergammaglobulinemia and anti-mitochondrial antibody, anti-smooth muscle antibody and anti-double stranded DNA antibodies	N/A	N/A	N/A	N/A	N/A	N/A	The patient diagnosed with auto immune hepatitis with primary biliary cholangitis overlap syndrome triggered by COVID-19
Seifert *et al* [44], 2023	N/A	7 patients (3 males & 4 females)	Post-COVID-19 cholangiopathy among 7 patients of 544 patients with cholangitis. 4 patients had SSC due to other reasons	Yes, elevated GGT, Alkaline phosphatase ALP among 7 patients more than 4 patients non COVID-19	N/A	N/A	N/A	N/A	N/A	N/A	The 7 patients with post-COVID-19 cholangiopathy showed more hepatitis and cholangitis than other group non-COVID cholangitis most probably due to direct cytopathologic effect of COVID virus
Lee *et al*[45], 2021	64	Male	Post-COVID-19 cholangiopathy	Yes	U/S intrahepatic bile ducts loss	MRI not available	Required MV	Yes, Recovered	Diffuse hepatic injury, onion skinning of the bile ducts and bile duct loss in scattered portal tracts	OLT not done; patient need to be stable for the operation	Not recovered
Cunha-Silva *et al*[46], 2023	45	Male	Post-COVID-19 cholangiopathy	Yes, elevated in the first 2-wk AST, ALT, GGT, Alkaline phosphatase post SARS-CoV-2 infection: ANA and anti-smooth muscle-positive. Negative viral hepatitis & anti-mitochondrial antibodies	N/A	No dilatation of biliary ducts	N/A	AKI after recovering 2 wk from COVID-19	Numerous foci of lobular necrosis but with no ductopenia or portal biliary reaction. After 2 mo: Biopsy showed: extensive areas of confluent necrosis,	N/A	The patient is given prednisolone in the first phase, then after 2 mo added azathioprine and UDCA to management

									hepatocytes regenerating into pseudorosettes and numerous plasma cells, non-suppurative cholangitis all these features diagnosed by PARIS Criteria as AIH-PBC-OS		and showed great response and recovery
Hamid *et al* [47], 2021	N/A	N/A	Post-COVID-19 cholangiopathy	Yes, elevated AST, ALT, low albumin, and low platelet	N/A	N/A	N/A	N/A	Endoscopy and ERCP are recommended by WGO	N/A	OLT is advised to be postposed till SARS-CoV-2 infection treated and patient recovered
Kroepfl *et al* [48]	N/A	2 patients	Post-COVID-19; cholangiopathy	Yes	N/A	N/A	N/A	N/A	ERCP biopsy showed severely destructed biliary mucosa with ischemia and epithelial roughness	N/A	N/A, early cholangioscopy can confirm the diagnosis
Mayorquín-Aguilar *et al* [24]	3 Cases 45 52 46	 Male Male Female	Post-COVID-19 cholangiopathy	Yes	Not available	Mild intrahepatic; Biliary ductal; Dilatation with; Multifocal strictures or; Beading without; Extrahepatic biliary; Dilatation	Yes, required MV	Yes, recovered	SSC-CIP beading of intrahepatic ducts, bile duct wall thickening with enhancement, and peribiliary diffusion high signal	2 Done OLT, 1 Not done	2 males death; 1 female recovered
Graciolli *et al* [49]	63	Male	Post-COVID-19 cholangiopathy	Yes	Not available	Dilations with intercalated stenotic segments in intra and extrahepatic bile ducts and edema of the bile ducts corresponding to inflammation of the adjacent parenchyma	Yes	Not available	Intrahepatocellular cholestasis	Not done	Death, infected ulcer, palliative care
Keta-Cov research group[50]	Median Age 59 (35-65)	Male Male Male Female	Post-COVID-19 cholangiopathy	Yes, elevated AST, ALTGGT, ALP, total bilirubin all elevated	N/A	Aspects of sclerosing cholangitis, with strictures and dilatations of intrahepatic bile ducts, peribiliary	All patients required M/V	All patients developed acute kidney injury required renal replacement therapy	ERCP showed filling defects in the common bile duct and rarefication of the intrahepatic biliary tract and biopsy showed	N/A	Intravenous ketamine is dose dependant and used for maintenance sedation of patients required

Zdanowicz *et al*[51], 2022	Paediatric patient	Female	Post-COVID-19 cholangiopathy	Yes	N/A	cysts and multiple biliary casts	N/A	N/A	biliary obstructions, including cholangiolar proliferation, biliary plugs, portal inflammation with neutrophil infiltrates, extensive biliary fibrosis and cirrhosis	N/A	M/V for acute respiratory distress syndrome ARDS, and showed associated with biliary obstructions, cholestatic liver injury, biliary cirrhosis, and end-stage liver disease, that's the reason the new guidelines is not recommend ketamine especially if prolonged or at higher dose
Schwarz *et al* [52], 2022	N/A	Male	Post-COVID-19 cholangiopathy	Yes, GGT is elevated in 15 patients with SSC-CIP after lung transplantation out of 40 patients in the study. ALP is elevated after lung transplant	N/A	N/A	15 patients out of 40 developed SSC-CIP underwent lung transplant	N/A	N/A	N/A	Patient developed autoimmune hepatobiliary diseases, autoimmune sclerosing cholangitis ASC which required long-term liver function monitoring
Keskin *et al* [53], 2022	N/A	15 patients	Post-COVID-19 cholangiopathy	Yes	N/A	N/A	N/A	N/A	N/A	N/A	GGT showed to be a sensitive parameter to predict severity in SSC-CIP
Bartoli *et al* [54], 2021	44	32 patients	Post-COVID-19 cholangiopathy	Yes, AST, ALT elevated and GGT, ALP elevated more ANA positive, anti-mitochondrial-positive, anti-smooth muscle negative	U/S showed slightly enlarged liver with moderate steatosis and a mildly enlarged spleen	N/A	Yes, required intubation and MV	N/A	N/A	Not done	Technical problems with ECRP were more common in biliary patients with delay group than in those without delay, while 7 pancreatic patients showed

											no difference in ERCP with or without delay of intervention. Technical issues considered such as abundant stone sludge in bile duct, stent migration, *etc*
Ferreira *et al* [55], 2022	N/A	Female	Post-COVID-19 cholangiopathy	Yes	N/A	N/A	N/A	N/A	Florid ductal lesions, moderate peri-portal fibrosis, portal chronic inflammatory infiltrate	N/A	Patient treated with UDCA and discharged and breathing normally, also treated from Guillain barre syndrome GBS by intravenous immunoglobulin
		4 cases				N/A			ERCP showed beaded appearance of intrahepatic bile ducts and bile casts		One patient undergone stone removal, and one patient complicate with liver cirrhosis, the other two progressed to advanced chronic liver disease
Bütikofer *et al*[56], 2021	N/A	20 Cases	Post-COVID-19 cholangiopathy	Yes 9 patients with severe cholestasis 11 patients with mild cholestasis	N/A	N/A	N/A	N/A	Ischemic changes to the perihilar bile ducts	N/A	SSC is more common and severe in critically COVID-19 patients, with prolonged ICU period
Zafar *et al* [57], 2022	N/A	2 Cases	Post-COVID-19 cholangiopathy	Yes	N/A	N/A	N/A	N/A	N/A	N/A	Both patients developed SSC post-COVID-19 vaccination, which lead to hepatitis and eventually cholangitis
Otani *et al* [58], 2022	N/A	N/A	Post-COVID-19 cholangiopathy in some cases of	Yes	N/A	N/A	N/A	N/A	N/A	N/A	166 cases for endoscopic

			166 cases								procedures for causes; Cholangitis, GI bleeding, Obstructive jaundice, neoplasia, COVID-19 led to delay in endoscopic procedures which led to delayed diagnosis of cholangitis, cancers, *etc*.
Cesar Machado *et al*[59], 2022	66	Male	Post-COVID-19 cholangiopathy	Yes	Ultrasound showed slight hepatomegaly and no bile duct dilatation	MRI showed biliary cast, also revealed. Diffuse irregularity of intra- and extrahepatic bile ducts, with multiple focal strictures alternating with mild focal dilations of the biliary tree, suggesting a sclerosing cholangiopathy	Yes, required MV	Yes, required haemodialysis	Biopsy showed a prominent bile ductular reaction, cholangiocyte injury, inflammatory infiltrate rich in neutrophils, biliary infarctions, marked cholestasis, and portal fibrosis	Not done OLT, due to poor clinical condition	Slight recovery, under observation & follow-up
Steiner *et al* [60], 2022	33	Female	Post-COVID-19 cholangiopathy	Yes, elevated liver enzymes AST, ALT, marked elevated GGT, ALP	N/A	MRCP done showed cholangiopathy	Yes hypoxia required intubation and MV, patient developed respiratory distress syndrome in which she was given veno-venous extracorporeal membrane oxygenation	Yes renal failure, and went through haemodialysis frequently	ERCP done showed cholangiopathy	OLT not done	Patient passed away, her clinical condition deteriorated, with severe hypoxia, renal failure, and multi-organ failure
Gourjault *et al*[61], 2021	55	Male	Post-COVID-19 cholangiopathy	Yes, elevated AST, ALT high GGT, ALP, elevated bilirubin, LDH	N/A	Periportal hypersignal without hepatic biliary dilatation	Yes, Required intubation& MV for 20 d with four sessions prone position	N/A	Interlobular biliary lesions with cholestasis	Waiting list for OLT	Discharged home, he had sphincterotomy and stone removal, planned for OLT

	45	Male			Hepatic steatosis without hepatomegaly or biliary dilatation	Diffuse intra-hepatic dilatation and liver steatosis without any focal obstructing lesion	MV for 26 d and sedated with ketamine for 24 d then he was on ECMO for 18 d	Fifteen sessions of hemodialysis			Discharged home, improved, not done OLT
	30	Male			US normal	Progressive irregular intrahepatic ductal dilatation	MV for 12 d with ketamine sedation, then replaced by ECMO for 29 d with 6 sessions of prone position	30 sessions of hemodialysis	Biopsy showed cholestatic hepatitis, bile ducts dystrophy	OLT done 11 mo after his admission	Developed liver failure with ascites, prolonged prothrombin, OLT done
Tafreshi *et al* [62], 2021	38	Male	Post-COVID-19 cholan-giopathy	Yes, mildly elevated AST, ALT and GGT mild bilirubin level	Intrahepatic biliary ductal irregularity and a markedly thickened common bile duct	Diffuse mild intrahepatic biliary distention, marked beading and irregu-larity& mild irregu-larity of the extra hepatic common bile duct	Required intubation & MV	N/A	Biopsy showed cholestatic hepatitis with cholangiocyte injury, bile ductular proliferation, canalicular cholestasis	Waiting list for OLT	Improved by treatment, waiting list for OLT
Leonhardt *et al*[63], 2023	N/A	N/A	Post-COVID-19 cholan-giopathy	Yes	N/A	N/A	Yes. Intubated on MV	N/A	N/A	N/A	One patient developed SSC-CIP in every 43 invasive ventilated COVID-19 patients (total 1082 patients)
Zengarini *et al*[64], 2022	30	Female	Post-COVID-19 cholan-giopathy	Yes	N/A	N/A	N/A	N/A	N/A	N/A	Patient developed subacute cutaneous lupus erythematosus post COVID-19 vaccination in patient with PBC
Wendel-Garcia *et al* [65], 2022	N/A	N/A	Post-COVID-19 cholan-giopathy	Yes. High total bilirubin	N/A	N/A	N/A	N/A	N/A	N/A	The study showed 243 patients; 170 Patients infused with ketamine developed post-COVID-19 cholangiopathy while other patients received propofol,

											fentanyl were not associated with cholestatic liver injury
Morão *et al* [66], 2022	46	Female	Post-COVID-19 cholangiopathy	Yes	N/A	MRCP; liver abscesses, intrahepatic bile duct dilation with multiple strictures and some linear repletion defects at the bifurcation of the common hepatic duct	Intubation with MV 12 d	N/A	ERCP Showed; biliary casts	N/A	N/A
Lee *et al*[67], 2022	56	Female	Post-COVID-19 cholangiopathy	Yes, hepatitis C, AST, 243, ALT 630, ALP 449, GGT 2765	N/A	N/A	N/A	N/A	Granulomatous cholangitis, nonsuppurative with destruction and proliferation of bile ducts with PBC Also immune infiltrations of CD3 T-cells, CD8 T-cells	N/A	Patient improved and discharged after high dose UDCA treatment, liver enzymes become normal
Nikoupour *et al*[68], 2020	35	Male	Post-COVID-19 cholangiopathy	Yes	N/A	N/A	N/A	N/A	N/A	OLT done before 3 yr from COVID-19 infection	Two identical twins had COVID-19 infection, both developed PSC, one of them who had OLT showed improvement with mild symptoms, while the other twin had more severe symptoms
	35	Male								Did not have OLT	
Arnstadt *et al* [69], 2021	62	N/A	Post-COVID-19 cholangiopathy	Yes	Echogenic intraductal longitudinal structures characteristic for intraductal casts and for SSC-CIP	MRCP showed irregular intrahepatic bile ducts	Yes, need long-term ventilation	N/A	Necrotic bile ducts	N/A	N/A
Meersseman *et al*[70], 2021	Mean age 48-68	Male	Post-COVID-19 cholangiopathy	Yes, elevated GGT, ALP, AST, ALT	N/A	MRCP showed focal strictures in intrahepatic bile ducts with	Yes, intubated & MV then VV-ECMO	Yes, required renal support	ERCP: Patient 1 diffuse beading of the intrahepatic biliary system,	OLT done for patient 1 and 2patient 3 & 4 did not	Patient 1 is doing well, patient 2 died due to septic

						intraluminal sludge and casts			patient 2 & 3 diffuse beading of the intrahepatic biliary ducts, patient 4 focal strictures on the right hepatic duct	undergo OLT	shock and pneumonia, patient 3 have mild SSC-CIP, patient 4 died due to lethal liver hemorrhage
Durazo *et al* [5], 2021	47	Male	Post-COVID cholangiopathy	Yes	Cholelithiasis without evidence of acute cholecystitis	Mild intrahepatic biliary ductal dilatation with multifocal strictures and beading with intra hepatic dilatation but without extrahepatic biliary dilatation	Yes, off MV on day 29	Yes, recovered	Liver abscess; Bile collection associated with bile duct dilatation with vacuolization and neutrophilia. Endothelial hepatic arteries swelling. Severe portal tract inflammation with Obliterative venopathy	OLT done	Recovered
Raes *et al* [71], 2022	64	Male	Post-COVID-19 cholangitis	Yes	N/A	N/A	Yes, MV then venovenous ECMO VV-ECMO	N/A	N/A	N/A	Passed away; patient having CAHA, progressive liver failure, secondary to ischemic cholangitis
Fajardo *et al* [72], 2021	24	Female	Post-COVID-19 cholangitis	Yes, GGT, ALP, AST, ALT, bilirubin	US: thickening of the gallbladder without stones	MRI: showed normal biliary tree and wall oedema of the gallbladder	N/A	N/A	Cholangitis of the small bile ducts consisting of a mixed inflammatory infiltrate with lymphocytes, plasma cells and neutrophils, accompanied by eosinophils, localized around and within the bile ducts	Not done OLT	Improved, patient discharged after laparoscopic cholecystectomy and liver biopsy
Pizarro Vega *et al*[73], 2023	63 66 60 65 44	Male Female Male Male Female	Post-COVID-19 cholangiopathy	Yes, GGT high in all patients especially in patient NO. 3 to 143 U/L then reached to 1130 U/L and patient 4 reached 3550 U/L. AST is high and higher in patient 4 to 82 U/L and patient 5 to 85 U/L then reached	N/A	MRI showed intrahepatic duct dilatations, stenosis without lithiasis, no extrahepatic duct alteration	Yes, required intubation, MV. Pronosupination	Yes, impaired renal function, required vasoactive drugs	No liver biopsy	One patient planned for OLT	All patients treated with UDCA and discharge. 3 patients re-admitted due to complication, patient 4 had pleural

Knooihuizen *et al*[74], 2021	68	Male	Post-COVID-19 cholangiopathy KISC	maximum 250 and 148, respectively. And patient 1 reached 1520 U/L. High tot. Bilirubin, ALT, ALP	N/A	MRI showed intrahepatic biliary dilatation with a beaded appearance & dilated common bile duct with distal narrowing	Yes	Yes	Liver biopsy showed minimal infiltration of neutrophils in the portal tract and lobule without cholestasis, also showed portal tract with bile duct injury	Not done	empyema. Patient 5 had cholecystectomy, patient 6 readmitted for acute cholangitis without lithiasis, no patients died during follow up
Zhou *et al* [75], 2022	54	Female	Post-COVID-19 vaccination leading autoimmune hepatitis	Yes, ALP peaked 2239 U/L, GGT 773 U/L, AST 1260 U/L, ALT 1729 U/L	N/A	N/A	N/A	N/A	Liver biopsy showed interface hepatitis with portal infiltration and discrete presence of rosette formation and apoptotic hepatocytes	Not done	Patient have KISC during intensive sedation, then ceased the KISC is transient, patient for repeat MRCP
Muehlenberg *et al*[76], 2021	36	Female	Post-COVID-19 cholangiopathy	Yes, AST 581, ALT 588 elevated, GGT, ALP slight elevation, bilirubin 1.4	US of liver and bile duct were normal	N/A	Yes, intubation and MV with antibiotics and catecholamine treatment	N/A	N/A	N/A	Patients have Autoimmune hepatitis AIH post vaccination (Moderna mRNA), treatment given after 2nd dose vaccine with prednisolone PSC treated with UDCA and ERCP
Soldera and Salgado[77], 2021	80	Female	Post-COVID-19 cholangiopathy	Yes, AST 100 U/L, ALT 113 U/L, bilirubin 12 mg/dL	Not Available	Diffuse irregularity of the intrahepatic bile ducts associated with sacular dilations suspicious for cholangiolithic abscesses	Yes	Not available	Intense cytoplasmic vacuolization of cholangiocytes and microvascular alterations	OLT done	Patient done ERCP with papillotomy and foreign body extraction
Rojas *et al* [78], 2021	62	Male	Post-COVID-19 cholangiopathy	Yes	Resemble SSC (secondary sclerosing cholangitis) but no portal inflammation, dilatation, or fibrosis)	MRI is negative	Yes, off MV on day 30	Yes, recovered	Low periportal inflammatory infiltrate without necrosis but with a severe obstructive cholestatic pattern	Not done	Recovery
Dhaliwal *et*	29	Female	Post-COVID-19 cholan-	Yes	Not Available	Mild intrahepatic	Not required	No	Filling defects	OLT Done	Not recovered

al[23], 2022			giopathy			biliary ductal dilation. With subtle central biliary enhancement concerning for. Cholangitis along with hypodense material hin extrahepatic. Biliary system likely representing transiting gallbladder. Sludge	MV		secondary to multiple large biliary casts (Biliary case syndrome)		
	42	Female		Yes							Recovery with long-term liability and comorbidity
Caballero-Alvarado *et al*[79], 2023	7 cases	7 cases	Post-COVID-19 cholan-giopathy	Yes	Not available	Not available	Not available	Yes, recovered	Secondary sclerosing cholangitis	1 done OLT 6 send for consideration of OLT	One recovered, 6 no data available
Soldera *et al l* [26], 2023	50	One male	Post-COVID-19 cholan-giopathy	Yes	Not available	MRI showed intra-hepatic sclerosing cholangitis and a dilated chilidium, with no signs of lithiasis (11 mm)	Yes, required MV	Yes, required haemodialysis	ERCP which showed a cast in the format of the external biliary tract, which was removed	Not done OLT	Recovered post cast removal
Franzini *et al* [80], 2022	65	Male	Post-COVID-19 cholan-giopathy	Yes	U/S showed no abnormalities	MRI Not available	Yes, required MV; under Fentanyl, Midazolam, and Ketamine sedation	Yes, required haemodialysis	ERCP revealed rarefaction of intrahepatic bile ducts, and removal of biliary casts	OLT Not done	No improvement
Roda *et al* [81], 2022	63	Male	Post-COVID-19 cholan-giopathy	Yes	Ultrasound results was inconclusive	MRI not done	Yes, required MV, and veno-venous extracor-poreal membrane oxygenation support (VV-ECMO). And eventually done bilateral lung transplant	Acute renal failure (AKI III); chronic illness neuropathy; several episodes of bacterial superinfections and lastly, PLS, characterized by severe haemolysis	Transjugular hepatic biopsy was performed with histopathological evidence of portal and periportal fibrosis, and intraparenchymal cholestasis with cholangiopathy and vasculopathy	OLT not done, patient did bilateral lung transplant	Not recovered, patient passed away due to Multiorgan failure MOF due to septic shock
Tebar *et al*	43	Male	Post-COVID-19 cholan-	Yes	Ultrasound	MRI not done	Yes, required	Not available	ERCP, MRCP	Not available	Not available

[82], 2022			giopathy		Not available		MV		Showed: Bile cholestatic, toxic. Cause necrosis of cholangiocytes and stenosis, determining persistent and irreversible biliary obstruction, with rapid progression to liver cirrhosis		
Santisteban Arenas *et al* [83], 2022	6 cases 55 54 62 56 73 34	 Male Male Male Female Female Male	Post-COVID-19 cholan-giopathy	Yes	Ultrasound not available	Destruction and curling of the pathway, beading of the intrahepatic bile duct	Yes, all 6 cases required MV	1 male not having renal failure or haemodialysis. All other 5 cases have renal failure; 2 females not required haemodialysis; 2 males required haemodialysis; 1 male have renal failure but not required haemodialysis	MRCP/ERCP showed destruction of biliary tract. In three of the six patients underwent liver biopsy, the most frequent findings were the presence of a reaction. Ductular, proliferation of cholangioles, infiltrate. Inflam-matory associated with the biliary epithelium with component. Lymphoplasmocyte and polymorpho-nuclear neutrophils	Not available	1 patient died, 5 other survived with severe comorbidities such as pneumonia, tracheal stenosis, pressure ulcers *etc*.

ACLD: Advanced chronic liver disease; AIH: Autoimmune hepatitis; AIH-PBC-OS: Auto-immune hepatitis-primary biliary cholangitis overlap syndrome; AKI: Acute kidney injury; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; ANA: Antinuclear antibodies; AST: Aspartate aminotransferase; CAHA: Cold agglutinin hemolytic anemia; CBD: Common bile duct; CBD: Common bile duct; CIP: Critically ill patients; CLD: Chronic liver disease; COVID-19: Coronavirus disease 2019; EUS: Endoscopic ultrasound; ECMO: Extracorporeal membrane oxygenation; ERCP: Endoscopic retrograde cholangiopancreatography; GI: Gastrointestinal; GGT: Gamma-glutamyl transpeptidase; IL: Interleukin; KISC: Ketamine-induced sclerosing cholangitis; MRI: Magnetic resonance imaging; MRCP: Magnetic resonance cholangiopancreatography; MV: Mechanical ventilation; N/A: Not applicable; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; OLT: Orthotopic liver transplantation; PBC: Primary biliary cirrhosis; PLS: Passenger lymphocyte syndrome; PSC: Primary sclerosing cholangitis; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; SSC: Secondary sclerosing cholangitis; TNF: Tumor necrosis factor; U/S: Ultrasound; UDCA: Ursodeoxycholic acid; WGO: World Gastroenterology Organization.

enzymes particularly alkaline phosphatase and gamma-glutamyl transpeptidase, and signs of liver dysfunction. Radiology showed bile duct thickening and enhancement, beading of the intrahepatic ducts, and peribiliary enhancement on diffusion. Additionally, every patient had severe respiratory distress syndrome and kidney failure reported as complications. Liver transplantation has been suggested as a potential management option for PCC, although its efficacy as a curative treatment requires further validation. Not all PCC patients require liver transplantation, as some may recover without undergoing this procedure. Studies have demonstrated that liver enzymes, especially alkaline phosphatase, total bilirubin, and gamma-glutamyl transferase, decrease after medical treatment of PCC. While liver transplantation is not suitable for all PCC patients, it remains the most effective option for select cases. Further research, clinical studies, and international collaborations are needed to gain a better understanding of this novel disease and

explore potential treatment avenues.

ARTICLE HIGHLIGHTS

Research background

The coronavirus disease 2019 (COVID-19) pandemic, declared by the World Health Organization in March 2020, has had devastating global impacts, resulting in millions of deaths and significant economic and humanitarian losses. Despite vaccination efforts, new variants of the virus continue to pose a threat, hindering control measures. While respiratory symptoms are common in COVID-19, extrapulmonary manifestations and derangement of liver enzymes have been observed. One emerging complication is post-COVID-19 cholangiopathy (PCC), characterized by bile duct inflammation and damage in recovered individuals. PCC presents with symptoms such as abdominal pain, fever, and jaundice, affecting both severe and milder cases. The prevalence and potential drug associations with PCC remain uncertain.

Research motivation

Understanding post-COVID-19 cholangiopathy is crucial due to its novelty and potential impact on recovered patients. Exploring the clinical presentation and management of PCC can provide valuable insights into its diagnosis and treatment. By addressing the knowledge gaps surrounding this condition, future research can develop effective strategies for patient care and improve outcomes in clinical practice. The significance of solving these problems lies in advancing our understanding of this novel disease and facilitating evidence-based approaches to manage post-COVID-19 cholangiopathy.

Research objectives

The primary objectives of this systematic review were to comprehensively analyze and synthesize existing evidence on post-COVID-19 cholangiopathy, focusing on the clinical presentation and management approaches documented in reported cases. By realizing these objectives, we provide a comprehensive overview of the current understanding of post-COVID-19 cholangiopathy, identify knowledge gaps, and contribute to the development of effective diagnostic and therapeutic strategies for this condition. The findings from this study can guide future research endeavors, leading to improved patient care and outcomes in the field of post-COVID-19 cholangiopathy.

Research methods

The research methods employed in this study adhered to the guidelines for preferred reporting items for systematic reviews and meta-analyses protocols. A comprehensive search was conducted in electronic databases (Scopus, Web of Science, and Medline/PubMed) using specified search terms. The search was limited to English, Spanish, and Portuguese language publications without any date restrictions. In addition to database searches, the reference lists of identified studies were manually searched. The inclusion criteria encompassed clinical case reports or case series focusing on post-COVID cholangiopathy, with detailed information on clinical presentation, diagnosis, management, and outcomes. Studies that lacked relevant clinical information or were unrelated to the topic were excluded. Two independent reviewers performed data extraction using a standardized form, and any discrepancies were resolved through discussion or consultation with a third reviewer. The extracted data included variables such as age, sex, clinical presentation, liver and renal function tests, imaging findings, histopathology, liver transplantation status, and outcomes. Data analysis involved descriptive techniques, including frequencies, means, and medians.

Research results

This systematic review identified a total of 540 patients with post-COVID-19 cholangiopathy, predominantly male (12.7%) and over 50-years-old (12.2%). Elevated liver enzymes were observed in nearly all patients during the acute phase (93.8), persisting in the chronic phase. Total bilirubin levels were elevated in 63.5% of cases, while alkaline phosphatase was 488 (90.3%) and gamma-glutamyl transferase levels consistently exceeded 1000 U/L. Imaging findings revealed biliary ductal dilatation with fibrosis on ultrasound in 41.6% of patients and bile duct thickening with contrast enhancement on MRI in 47.7% of patients. Respiratory failure type 2, associated with acute respiratory distress syndrome, was observed in 41.3% of patients, with 1 patient undergoing lung transplantation. Acute renal injury requiring dialysis or renal transplantation was present in 65.7% of cases. Liver biopsy showed sclerosing cholangitis in 74.4% of patients. Sixteen patients (2.96%) underwent orthotopic liver transplantation, with successful outcomes observed in 93.75% of these cases. These findings provide important insights into the clinical characteristics and complications of post-COVID-19 cholangiopathy, highlighting the need for further research to elucidate its pathogenesis and optimal management strategies.

Research conclusions

This study proposes several new theories and methods in the field PCC. First, the study suggests that PCC is a serious systemic illness that affects not only the lungs but also the liver. It provides evidence that PCC is characterized by elevated liver enzymes, biliary ductal dilatation, and histopathological findings of secondary sclerosing cholangitis. The study highlights the importance of considering the differential diagnosis, as other diseases may present with similar symptoms, such as ketamine-induced cholangiopathy and ischemic cholangitis. The study emphasizes the diagnostic procedures for PCC. It recommends the use of cholangiography, endoscopic retrograde cholangiopancreatography, or

magnetic resonance cholangiopancreatography to visualize the biliary system and identify characteristic features of PCC, such as pruned tree appearance, beaded ducts, and band-like strictures.

Research perspectives

The future research in the field of PCC should focus on understanding its pathophysiology, including the mechanisms of bile duct paucity and unique microvascular features. Improving diagnostic procedures through novel imaging techniques and biomarkers is essential for early and accurate detection. Comparative studies with other cholangiopathies can enhance treatment approaches. Additionally, investigating the management and treatment of PCC, including the efficacy of liver transplantation, is crucial. Identifying predictive factors for transplantation and determining long-term prognosis are valuable areas of research. Overall, future studies should deepen our understanding, develop improved diagnostics, and explore effective treatments to enhance patient outcomes. Collaboration among researchers and international efforts will play a vital role in advancing knowledge and management of this disease.

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FOOTNOTES

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Country/Territory of origin: United Kingdom

ORCID number: Vinícius Remus Ballotin 0000-0002-2659-2249; Lucas Goldmann Bigarella 0000-0001-8087-0070; Jonathan Soldera 0000-0001-6055-4783.

Corresponding Author's Membership in Professional Societies: Federação Brasileira De Gastroenterologia; Sociedade Brasileira de Hepatologia.

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SYSTEMATIC REVIEWS

Potential long-term neurological and gastrointestinal effects of COVID-19: A review of adult cohorts

Zaki A Sherif, Mrinalini Deverapalli, Suryanarayana Reddy Challa, Zara Martirosyan, Peter Whitesell, Antonio Machado Pizuorno, Zainab Naqvi, Ingrid K Tulloch, Gholamreza Oskrochi, Hassan Brim, Hassan Ashktorab

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Zaki A Sherif, Department of Biochemistry & Molecular Biology, Howard University College of Medicine, Washington, DC 20059, United States

Mrinalini Deverapalli, Howard University Cancer Center, Howard University, Washington, DC 20060, United States

Suryanarayana Reddy Challa, Zara Martirosyan, Department of Internal Medicine, Howard University Hospital, Washington, DC 20060, United States

Peter Whitesell, Department of Pulmonary Disease/Sleep Medicine, Howard University Hospital, Washington, DC 20060, United States

Antonio Machado Pizuorno, Department of Internal Medicine, The University of Texas Health Science Center at Houston, Houston, TX 77030, United States

Zainab Naqvi, Medical Center, Georgetown University, Washington, DC 20007, United States

Ingrid K Tulloch, Department of Psychology, Morgan State University, Baltimore, MD 21251, United States

Gholamreza Oskrochi, College of Engineering and Technology, American University of the Middle East, Kuwait Egaila 54200, Kuwait

Hassan Brim, Pathology & Cancer Center, Howard University College of Medicine, Washington, DC 20060, United States

Hassan Ashktorab, Medicine & Cancer Center, Howard University College of Medicine, Washington, DC 20060, United States

Corresponding author: Zaki A Sherif, PhD, Full Professor, Department of Biochemistry & Molecular Biology, Howard University College of Medicine, 520 W Street NW, Washington, DC 20059, United States. zaki.sherif@howard.edu

Abstract

BACKGROUND

The respiratory infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has evolved into a multi-organ disorder, with long-term effects known as post-acute sequelae of SARS-CoV-2 infection or long coronavirus disease (COVID).

AIM
To examine the current knowledge and outcomes of long-term neurological and gastrointestinal (GI) symptoms in adult cohorts, including United States minority populations.

METHODS
PubMed and Google Scholar were searched using relevant terms, and data from five studies were analyzed, comprising 27383 patients with persistent neurological and GI sequelae.

RESULTS
The main symptoms included anxiety, depression, dysphagia, headache, vomiting, nausea, gastroesophageal reflux, fatigue, and abdominal pain. Patients with comorbidities and metabolic syndromes were at higher risk for long COVID. While most patients were European Americans, there was a need for further study on African Americans.

CONCLUSION
The underlying causes of these symptoms remain unclear, warranting more investigation into the long-term impact of the SARS-CoV-2 on different populations.

Key Words: Angiotensin converting enzyme; Long coronavirus disease; Post-acute sequalae of SARS-CoV-2 infection; Neurological; Gastrointestinal; Post-viral syndromes

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Core Tip: Long coronavirus disease (COVID) or post-acute sequelae of severe acute respiratory syndrome coronavirus 2 infection (PASC) can lead to prolonged and debilitating symptoms beyond 30 d after infection. Neurological manifestations are prevalent, with encephalopathy, myalgia, headache, and anosmia being common symptoms. Females seem to be more susceptible to long COVID, and severe disease is associated with longer or more frequent neurological symptoms. Gastrointestinal (GI) sequelae are also reported, with symptoms like difficulty swallowing, nausea, vomiting, and abdominal pain being common. Anxiety, depression, dysphagia, headache, and fatigue are among the top symptoms observed, with potential neurological and GI associations. However, there is a need for further research to explore the underlying causes and potential discrepancies in symptom reporting among different populations affected by long COVID/PASC.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is not only a respiratory illness; it can lead to multi-organ complications. Studying the long-term effects helps identify the post-acute sequelae of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (PASC) or long COVID (LC), which may involve neurological and gastrointestinal (GI) symptoms. Recognizing these sequelae is vital for developing targeted interventions and treatments. Among the wide range of COVID-19 patients who experience mild or severe symptoms, there is a subset that sustains a prolonged residual illness that lasts beyond 4 wk. Barring the lack of accurate diagnostic or reporting methods and normalized data for age and sex across countries, global estimates of LC or PASC patients vary widely between 15% to 76% depending on the preexisting medical conditions and geographical locations. Irrespective of acute COVID-19 severity and age, reports from the globally affected populations show that neurological and GI[1] dysfunctions remain as the most significant alterations. Post-infectious fatigue syndrome linked to brain fog and post-exertional malaise is not rare. Most well-studied viral or bacterial pathogens have been connected to the development of chronic symptoms in a subset of infected patients such as myalgic encephalomyelitis/chronic fatigue syndrome and postural orthostatic tachycardia syndrome[2-5].

LC or PASC is a multifactorial condition that lingers in many COVID-19 patients for more than 30 d following the initial viral infection that precipitates COVID-19. This residual illness displays over 200 symptoms and affects multiple tissues, organs, and biological systems including neuropsychiatric and GI systems to varying degrees[6-8]. The major symptoms of PASC are fatigue, brain fog (cognitive dysfunction), post-exertional malaise, and shortness of breath (dyspnea) that can last for months and can debilitate normal daily activities. There are several hypotheses that attempt to explain the underlying mechanisms that propel these multifaceted symptoms. Some of the leading theories include viral persistence, chronic inflammation, autoimmune dysfunction, and microclot formation[9]. In most of the tissues and organs affected by LC/PASC including the neurological and GI disorders, there is evidence of endothelial dysregulation.

This has prompted some scientists to declare that PASC is a vascular disease.

There are no universal case definitions of PASC. The National Institute of Health (NIH) defines PASC as "the failure to recover from acute COVID-19, or those persistently symptomatic for > 30 d from onset of infection, with any pattern of tissue injury that remains evolving including the nervous system" (www.NIH.gov). The Centers for Disease Control defines PASC as a condition marked by the continuation of COVID-19 symptoms for four or more weeks after infection with SARS-CoV-2 (www.CDC.gov). The SARS-CoV-2 has been detected in brain tissue and the GI tract. Early research indicates that the virus enters the brain through the nose, and *via* the olfactory bulb invades the brain cells (neurons) where it prowls unchecked, conceivably leading to lasting neurological symptoms, such as cognitive impairment and brain fog[2,10-15]. The virus can also invade the GI system through angiotensin-converting enzyme 2 (ACE2). Understandably, the GI infiltration of the virus begins in the oral route and may precede the brain invasion. It is not clear, however, how the virus precipitates the neurological or GI symptoms that are common in LC patients. The postulation, although not universally accepted by researchers or scientists, is that neuronal inflammation resulting from the viral invasion and persistence in these biological systems may be the trigger for the GI and neurological symptoms[2,10-13,16].

Understanding the full spectrum of COVID-19 is essential to provide appropriate medical care and support to patients as the disease is relatively new and its long-term effects are still being discovered. By reviewing the existing literature, scientists can gain a comprehensive understanding of the various neurological and GI symptoms that can persist after the acute phase of the infection. In the general COVID-19 population of the United States, those with neurological and GI symptoms experience viral invasion in the central nervous system through vascular and lymphatic systems or the vagal nerve[17]. SARS-CoV-2 or its viral particles including the RNA or the associated proteins can infect leukocytes and migrate into the brain or can be directly transported across the blood-brain barrier (BBB) to the brain. Furthermore, ongoing research reports that the virus can invade the peripheral lymphatic vessels that connects the glymphatic system of the brain[18]. The sequential correlation between neurological and GI symptoms or disorders lends credence to the vascular system as being the primary culprit. But the lymph vessels around the GI tract, or the gut-brain axis or the enteric nervous system may also facilitate entry for SARS-CoV-2 to the brain[19,20].

Minority patients including African Americans and Hispanic Americans have been disproportionally affected by COVID-19. These two population groups share common symptoms with most of COVID-19 patients. However, there is a dire need to characterize the long-term effects and impact of these neuropsychiatric and GI symptoms as a significant proportion of these populations usually are socially and economically poor, and medically underserved. African Americans and Hispanic Americans have a higher disease burden and fatality rates from COVID-19 than the general population. Studies regarding LC sufferers in these communities are rare and the long-term outcomes are difficult to gauge based on the available public data. It is even rarer to assess the neurological and GI tract symptoms in such patients because their asymptomatic and mild cases are scarcely documented. Therefore, although the neurological and GI symptoms may be shared by most SARS-CoV-2-infected long haulers, there must be particular attention afforded to those disproportionally affected patients to truly understand the underlying biological and pathophysiological mechanisms of PASC under a different social construct.

In the United States, there is a high frequency of neurologic involvement in 82% of hospitalized COVID-19 patients [21]. However, most neuro-COVID-19 clinic population consists of individuals who were never hospitalized for respiratory complications resulting from COVID-19, and this includes primarily minority populations. Studying the enduring neurological and GI symptoms in minority LC patients who suffered disproportional affliction during the pandemic, which received scant research in the literature, is crucial. Although other combinations of symptoms such as neurological and pulmonary symptoms or their variations are also apparently important to study, there are numerous reviews on this subject. Comprehensive research in various symptom domains contributes to a holistic understanding of COVID-19 and its effects on human health. In this retrospective review, we will characterize the cardinal symptoms shared or exhibited by PASC patients particularly from communities that are underserved and disproportionally affected by the coronavirus pandemic.

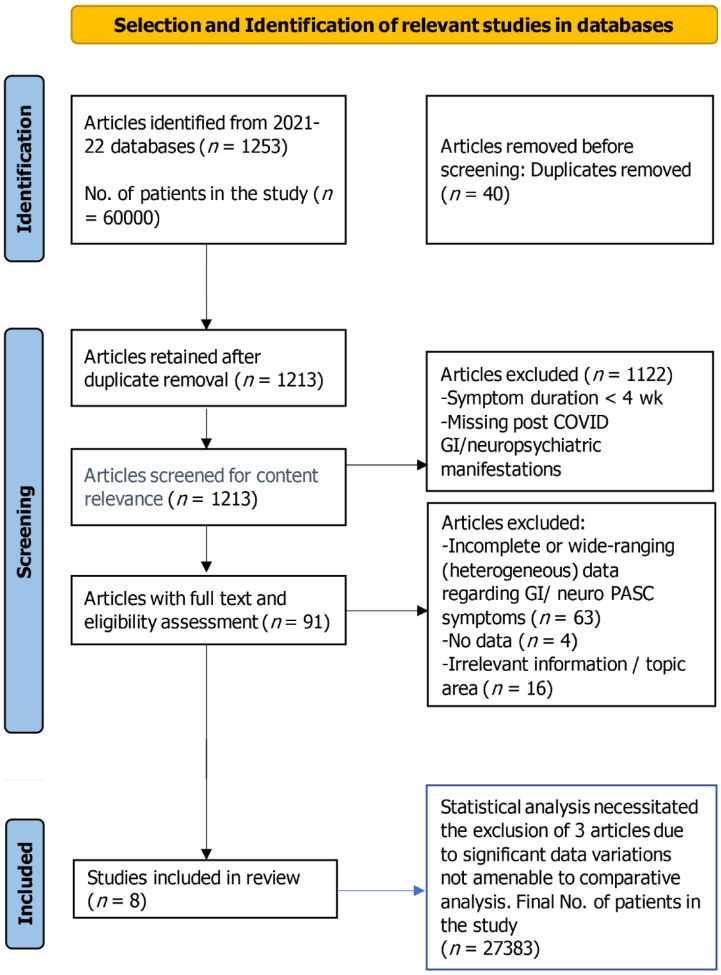
MATERIALS AND METHODS

Search strategy and selection criteria

We conducted a systematic literature search of published articles using PubMed and Google Scholar databases from December 2019 to September 30, 2022. We used the following search terms: COVID-19, ACE2, angiotensin converting enzyme, and SARS-CoV-2, long COVID or PASC (post-acute sequalae of SARS-CoV-2 infection), neurological, GI, gastric sequelae of SARS-CoV-2, COVID-19, and post-viral syndromes in the United States. The protocol of this systematic review and analysis of COVID-19 patients' data is in accordance with the PRISMA Statement guidelines (https://prisma-statement.org) (Figure 1).

Inclusion and exclusion criteria

We first sorted the LC/PASC studies by title and abstract; then, we compiled the papers by relevance and conducted a new selection process by thoroughly reviewing the data. We incorporated studies that reported GI and neuropsychiatric findings in COVID-19 patients and long-haulers. From the selected papers, tables were generated for each data set in Microsoft Excel. These tables included the following information for each study (when available): General information about the study (year, location, hospital or city, state and country, and publication date), confirmed cases, GI/neuropsychiatric manifestations, symptoms involving other organ systems, comorbidities, hospitalization, outcome measurements, and PASC duration. We compared clinical manifestations, comorbidities, prevalence, and significant outcomes of the



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Figure 1 Selection and identification of relevant studies in databases. COVID: Coronavirus disease; PASC: Post-acute sequelae of severe acute respiratory syndrome coronavirus 2 infection; GI: Gastrointestinal.

long-term neurological and GI sequelae of COVID-19 symptoms. We particularly attempted to include United States minority populations where available.

Inclusion criteria

The following inclusion criteria were adopted to validate article selection: Any study including patients with confirmed previous COVID-19 diagnosis with specified post-COVID neurological and GI sequelae of COVID-19 findings; any study with five or more patients; any study with all or most patients from Western countries including United States minority populations with no distinction regarding sex, age, severity of disease, inpatient or outpatient management, treatment, and outcome. Data on neurological and GI symptoms after 4 wk of SARS-CoV-2 infection were collected and presented.

Exclusion criteria

The following exclusion criteria were adopted to filter out incomplete data: Studies whose duration of symptoms following SARS-CoV-2 infection lasted less than 4 wk were excluded. In addition, studies which did not include post-COVID GI/neuropsychiatric manifestations and studies from Eastern countries were also excluded to have a more homogeneous data source.

Statistical analysis

The potential studies were assessed for data homogeneity including demographics and LC symptoms. Finally, five studies were included in the analysis of this review. The statistical analysis was performed, as appropriate, by weighted analysis where the weights were associated with the size of the study and the inverse of the variance of the main outcome. Descriptive, parametric, and non-parametric correlation, Chi-square tests, and *t*-tests, were performed using SPSS and Excel as appropriate. The primary limitation of this review, as our biostatistician has determined, is that the data are aggregated and confined to a small number of papers. Therefore, correlation between GI and neurological symptoms may not be robust.

RESULTS

Baseline patient characteristics and symptoms

More than 60000 COVID-19 patients of aggregate studies with various COVID and LC symptoms were initially screened for this review. Dozens of papers were sorted out for specific data on LC or PASC symptoms lasting more than 30 d. After excluding duplicates and unrelated studies by screening the title, abstract, or main text, a total of five published studies were included in this review containing a total of 27383 patients with long-term neurological and GI sequelae of COVID-19 symptoms. The mean age of these cohorts is 55.76 years. Females outnumber males by 1.6 to 1.0 as shown in Table 1 and Figure 2.

The baseline demographics of the aggregate study cohorts include European Americans (66.5%), African Americans (9.9%), Asian Americans (3.0%), Hispanic Americans (0.5%), and persons with undeclared (or unrecorded) racial or ethnic identities (20.0%), which made up the second largest group (Figure 2).

Table 2 and Figure 3 show PASC patients harboring GI or neurological symptoms, or both as documented in the five articles selected for this systematic review.

The papers did not single out PASC patients with overlapping GI and neuropsychiatry symptoms. Table 2 also displays the minimum and maximum numbers of each symptom reported among the five studies. Sometimes, only one or two papers reported certain symptoms. For example, among the GI symptoms, dysphagia (*i.e.*, difficulty swallowing) was the number one cited symptom (self-reported by 6114 PASC patients) in one of the studies (Table 2, Figure 3). However, this symptom was cited in only two of the three papers. Similarly, anxiety, the highest-ranked neurologic symptom by 6749 PASC patients, was cited by only two of the papers (Table 2). The separation of GI and neurologic symptoms into Figure 4A and B, respectively provides a clearer picture of the most common symptoms associated with each disorder.

GI symptoms

Various GI symptoms to a different degree were reported in the five studies that are reviewed (Table 3, Figure 4A).

These common symptoms in order of descending rank in the aggregated data included abdominal pain (3718, 3 studies)[22-24], dysphagia (6114, 2 studies)[24-27], nausea/vomiting (5223, 3 studies), gastroesophageal reflux (4858, 1 study)[24], and constipation (3116, 2 studies)[23,24]. The remaining GI symptoms were diarrhea (2711, 4 studies)[22-24, 26], general GI (1290, 1 study)[24], anorexia (1259, 2 studies), and dyspepsia (5, 1 study).

Neurological symptoms

There are more neurologic symptoms reported than GI symptoms. The five most common neurologic symptoms in the aggregated data of the studies are: Anxiety (6749, 2 studies), depression (6292, 3 studies), headache (5295, 5 studies), myalgia (5076, 4 studies), and fatigue (4038, 5 studies). These top five and the remaining neurologic symptoms are shown in Table 4 and depicted in Figure 4B.

Comorbidities

The five most reported pre-existing conditions or comorbidities by 1266 PASC patients in the study cohorts were documented by four of the five papers reviewed (Table 5). Females constituted about 61% of COVID-19 patients more likely to experience PASC than males. In the cumulative study cohort, those reporting PASC symptoms consisted of White/Caucasians at 66.54%, African Americans at 9.97%, and Asians at 3.0% (Table 1). The leading comorbidities are hypertension (617, 4 studies), diabetes (343, 4 studies), obesity (320, 3 studies), asthma (172, 4 studies), and hyperlipidemia (145, 2 studies)[23,26]. The remaining comorbidities are shown in Table 5 and Figure 4C.

General symptoms

In Table 6, various symptoms other than GI and neurologic were reported by all five studies to varying levels. Furthermore, in the same five study populations, a total of 57 clinical manifestations were also assessed. We divided these clinical manifestations into separate categories of organ systems such as neurologic/psychiatry, mental health, respiratory, cardiovascular, GI, dermatologic, and ear, nose, and throat. The highest ranked general PASC-associated symptom by far was weight gain at 11256 followed by dyspnea at 5638. The rest of these general symptoms are side effects of SARS-CoV-2 infection and are listed in Table 6.

DISCUSSION

COVID-19 patients experience a multitude of symptoms including respiratory, digestive, and neurological disorders. A subset of these patients experiences LC or PASC that prolongs the duration and the debilitating conditions of these symptoms beyond 30 d after infection. As there is no standard case definition for PASC, there are disparate PASC explanations ranging from a minimum of 30 d duration of symptoms to a maximum of 6 mo or 180 d in the study cohorts. Certain distinctive symptoms such as anosmia indicate a potential neurotropism of this virus. There are several pathways for the virus to enter the nervous system. One of these pathways proposed by Llorens *et al*[28], is a route to the brain from the infection of the gut *via* Toll-like receptor 4 and zonulin brain receptor. Other researchers proposed that the evolutionary similarity of SARS-CoV-2 with SARS-CoV makes it more likely that SARS-CoV-2 can invade the olfactory bulb and GI system through ACE2[18]. However, when the GI tract is invaded, the virus may enter the central nervous

Table 1 Baseline demographics of post-acute sequelae of severe acute respiratory syndrome coronavirus 2 study cohorts

Ref.	[22]	[23]	[24]	[25]	[26]	Aggregate	%
Female	236	174	16177	104	71	16762	61.21%
Male	294	190	9940	85	112	10621	38.79%
Mean age (SD), year	59.2	61.0	51.6	50.0	57.0	54.3	-
African American	62	76	2551	20	16	2725	9.97%
European American	155	74	17752	148	99	18228	66.54%
Asian American	79	6	704	14	16	819	3.00%
Hispanic	132	0	0	0	0	132	0.48%
Unknown	102	208	5110	7	52	5479	19.98%

Table 2 Gastrointestinal and neurological post-acute sequelae of severe acute respiratory syndrome coronavirus 2 symptoms

Symptom	Number of studies	Minimum number	Maximum number	Total of 5 studies
Anxiety	2	11	6738	6749
Depression	3	6	6268	6292
Dysphagia	2	3	6111	6114
Headache	5	6	5223	5295
Nausea and/or vomiting	3	3	5197	5223
Myalgia	4	11	4962	5076
Gastroesophageal reflux	1	4858	4858	4858
Fatigue	5	29	3839	4038
Abdominal pain	3	2	3682	3718
Dizziness or vertigo	2	7	3656	3663
Constipation	2	8	3108	3116
Stress	1	2925	2925	2925
Neuropathy	2	27	2794	2821
Paresthesia	2	2	2794	2796
Diarrhea	4	2	2690	2711
Abnormal gait	2	18	1540	1558
Brain fog	5	16	1410	1489
Smell & taste problems	4	8	1305	1356
General GI	1	1280	1280	1280
Anorexia	2	5	1254	1259
Dyspepsia	1	5	5	5

GI: Gastrointestinal.

system through vascular and lymphatic systems or the vagal nerve. SARS-CoV-2 can infect leukocytes and migrate with them into the brain, or the viral particles can be directly transported across the BBB to the brain.

At the beginning of the pandemic in 2019, there have been frequent neurologic manifestations and encephalopathy-associated morbidity in COVID-19 patients. In fact, more than 80% of hospitalized COVID-19 patients had neurologic symptoms during their disease course, and 82.3% at any time during the disease course according to a retrospective Chicago-area study[21]. In this study, in order of decreasing percentages of neurological symptoms, myalgia (44.8%), headache (37.7%), encephalopathy (31.8%), dizziness (29.7%), dysgeusia (15.9%), and anosmia (11.4%) were the most frequent neurologic manifestations[21]. These neurological symptoms were more prominent in the Chicago-area cohort than the COVID-19 symptoms observed in the adult cohort populations studied in the five papers reviewed in our study

Table 3 Gastrointestinal post-acute sequelae of severe acute respiratory syndrome coronavirus 2 symptoms

Symptom[1]	Number of studies	Minimum number	Maximum number	Total of 5 studies
Dysphagia	2	3	6111	6114
Nausea and/or vomiting	3	3	5197	5223
Gastroesophageal reflux	1	4858	4858	4858
Abdominal pain	3	2	3682	3718
Constipation	2	8	3108	3116
Diarrhea	4	2	2690	2711
General GI	1	1280	1280	1280
Anorexia	2	5	1254	1259
Dyspepsia	1	5	5	5

[1]Patients may harbor more than one symptom.
GI: Gastrointestinal.

Table 4 Neurological post-acute sequelae of severe acute respiratory syndrome coronavirus 2 symptoms

Symptom[1]	Number of studies	Minimum number	Maximum number	Total of 5 studies
Anxiety	2	11	6738	6749
Depression	3	6	6268	6292
Headache	5	6	5223	5295
Myalgia	4	11	4962	5076
Fatigue	5	29	3839	4038
Dizziness or vertigo	2	7	3656	3663
Stress	1	2925	2925	2925
Neuropathy	2	27	2794	2821
Paresthesia	2	2	2794	2796
Abnormal gait	2	18	1540	1558
Fever	3	2	1463	1478
Brain fog	5	16	1410	1489
Smell & taste problems	4	8	1305	1356

[1]Patients may harbor more than one symptom.

demonstrating that anxiety (Table 2) was the most prevalent GI-neuro symptom. In another cohort of LC sufferers reported by Nakhli *et al*[29], depression (65%), but not anxiety (48%), was significantly more common in those with post-COVID-19 disorders of gut-brain interaction.

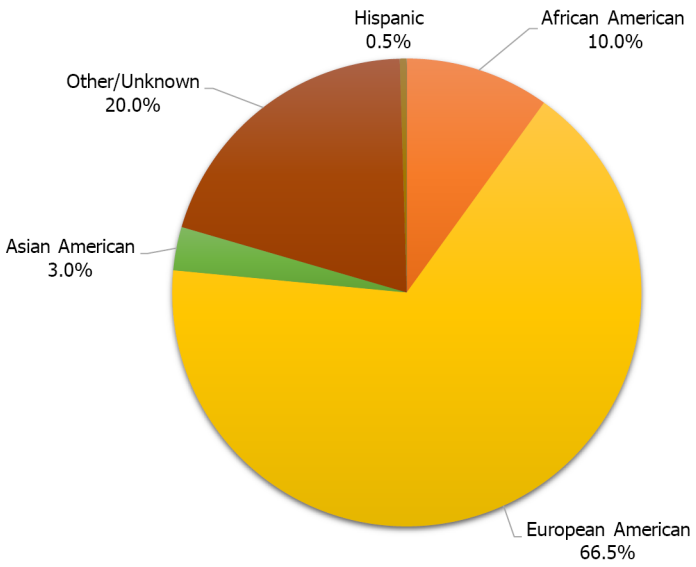
The five studies in our report did not categorize PASC patients based on the timing of neurologic manifestations by COVID-19 severity. The mean age was 55 years included female cohort of 62% compared to 38% of the male PASC patients raising the question as to why females seem to be more susceptible to LC. Four of our five study cohorts also comprised 645 hospitalized COVID-19 patients who later developed PASC. The literature is replete with data of PASC patients who developed their various residual illnesses following a lengthy stay in intensive care units connected to ventilators.

As Table 4 summarizes, the neurologic manifestations that occurred at onset and any time during COVID-19 show that patients with severe disease had a longer duration or frequency of neurologic manifestations including encephalopathy than those with milder or no symptoms[21]. The severity of COVID-19 symptoms can be influenced by genetic factors such as variations in the expression of the ACE2 receptor in the body, as well as differences in the virulence and transmissibility of the virus strains[30]. It is also evident in the evolving PASC literature that among the most reported neurological symptoms is fatigue. However, in our limited systematic review, this common condition was not among the top five neurological symptoms. Fatigue is also a major symptom among COVID-19 patients regardless of whether the

Table 5 Comorbidities in post-acute sequelae of severe acute respiratory syndrome coronavirus 2 patients

Ref.[1]	[22]	[23]	[25]	[26]	Aggregate sum
Total (*n*)	530	364	189	183	1266
Hypertension	266	225	39	87	617
Diabetes	146	134	11	52	343
Obesity	158	-	72	90	320
Asthma	55	74	24	19	172
Hyperlipidemia	-	125	-	20	145
CAD	52	41	3	21	117
Heart failure	23	41	-	5	69
Anxiety disorder	-	-	54	-	54
Mood disorder	-	-	43	8	51
CVA	28	21	-	-	49
Cancer	15	32	-	-	47
Chronic kidney disease	-	29	-	-	29
Smoking	18	-	9	-	27
COPD	19	-	-	7	26
Other	-	-	15	-	15
HIV	10	-	4	-	14
Atrial fibrillation	-	-	2	9	11
Valvular heart disease	-	-	3	-	3

[1]Patients may harbor more than one symptom.
CAD: Coronary artery disease; CVA: Cerebral vascular accident; COPD: Chronic obstructive pulmonary disease; HIV: Human immunodeficiency virus infection.



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Figure 2 Aggregate racial identities of the study cohorts.

Table 6 Other general symptoms associated with post-acute sequelae of severe acute respiratory syndrome coronavirus 2

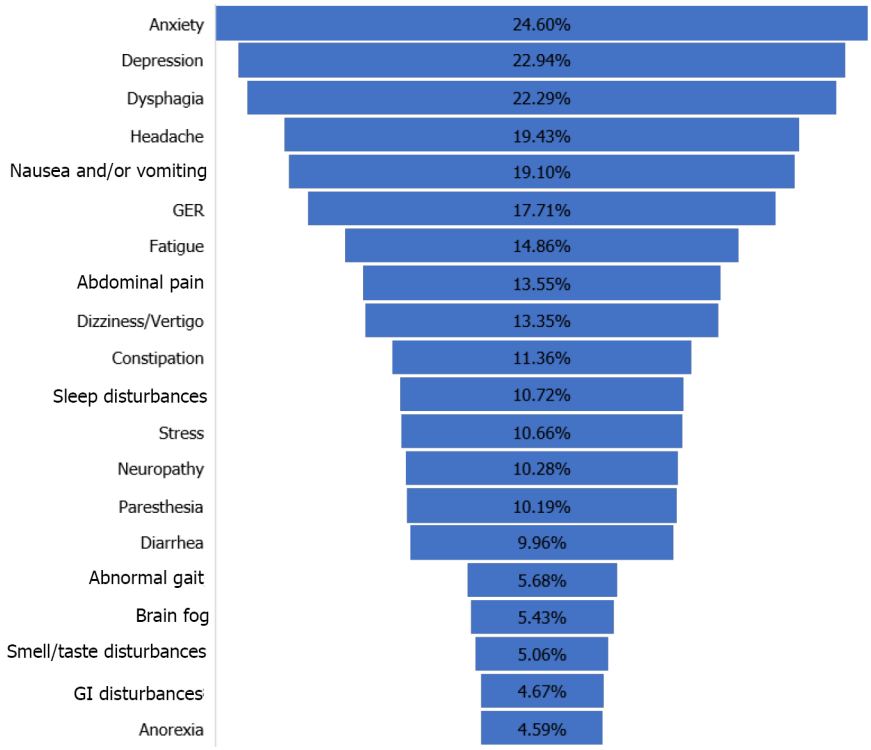
Ref.	[22]	[23]	[24]	[25]	[26]	Aggregate sum
Total number	530	364	26117	189	183	27383
Weight gain	-	-	11256	-	-	11256
Dyspnea	55	58	5432	35	58	5638
Joint pain	-	-	5484	6	29	5519
Cough	10	37	4570	-	46	4663
Chills	-	-	3839	-	-	3839
Edema	-	-	3839	-	-	3839
Bleeding	-	-	3708	-	-	3708
Tachycardia	-	-	3264	16	-	3280
Pain	-	-	3212	-	-	3212
Wheezing	-	-	3108	-	-	3108
High BP	-	-	3108	-	-	3108
Skin lesion	-	-	2977	-	-	2977
Sleep disturbances	-	-	2925	17	-	2942
Swelling	-	-	2742	-	-	2742
Chest pain	7	30	2690	10	-	2737
Rash	-	-	2455	-	-	2455
Erythema	-	-	2403	-	-	2403
Urinary tract symptoms	-	-	2272	-	-	2272
Weakness	-	34	2167	1	-	2202
Peripheral edema	-	22	2063	-	-	2085
Weight loss	-	-	1906	-	-	1906
Erectile dysfunction	-	-	1854	-	-	1854
Sinonasal congestion	-	-	1671	-	-	1671
Respiratory distress	-	-	1515	-	-	1515
Sleep apnea	-	-	1410	-	-	1410
Throat pain	-	-	-	11	-	11
Tinnitus	-	-	5.2	-	-	5.2
Nasal congestion	-	-	-	3	-	3
Hearing loss	-	-	-	2	-	2
Sore throat	-	-	-	-	-	0
Alopecia	-	-	-	-	-	0

BP: Blood pressure.

disease progresses to LC or not. A notable factor is that the most frequently observed LC symptoms in our meta-analysis are anxiety and depression, which are also features of post-traumatic stress disorder (PTSD).

The GI sequelae of SARS-CoV-2 can affect any part of the digestive system, not only in the acute infection phase but also in the post-acute phase, leaving long-term sequelae to manifest frequently or sporadically. The main long-term symptoms that are reported, regardless of the presence of chronic diseases, are diarrhea, nausea, vomiting, abdominal pain, accompanied by increased liver enzymes[31]. In our retrospective study of 27383 PASC patients, the main GI symptoms experienced in decreasing order are difficulty swallowing, nauseas and/or vomiting, abdominal/or visceral pain, GI reflux, diarrhea, and constipation.

Wang *et al*[24] developed a broad post-acute sequelae of SARS-CoV-2 symptoms lexicon called PASCLex based on physician clinical notes and reviews to facilitate PASC symptom identification and research. There were multiple

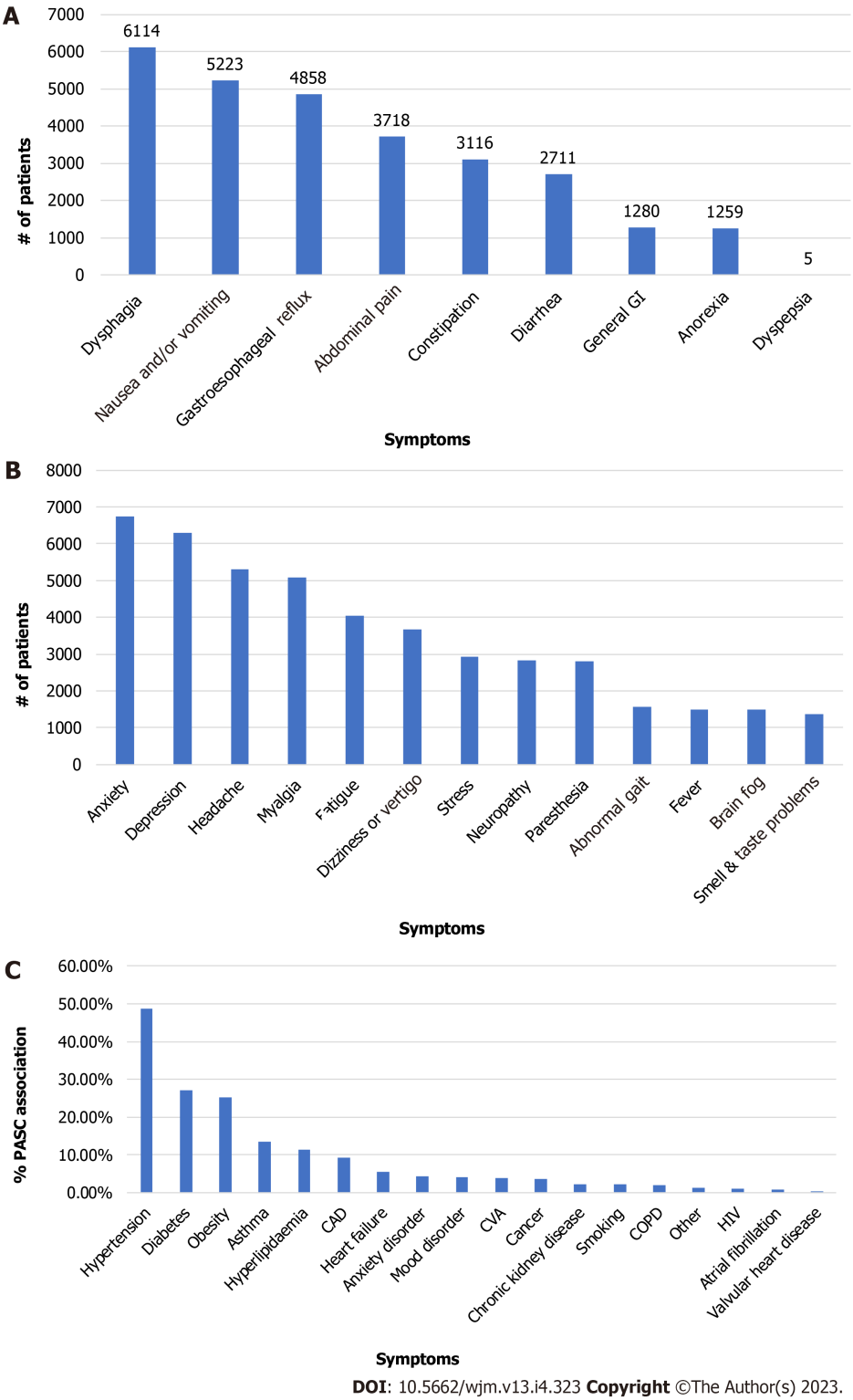


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Figure 3 Percentages of post-acute sequelae of severe acute respiratory syndrome coronavirus 2-gastrointestinal/neurological symptoms in all patients of the five studies reviewed. Patients with neurological or gastrointestinal symptoms may or may not harbor both symptoms. GI: Gastrointestinal; GER: Gastroesophageal reflux.

symptoms identified by the natural language processing tool that Wang *et al*[24] utilized to validate previously identified post-acute COVID-19 results recorded in meta-analysis studies of observational and survey data. Lopez-Leon *et al*[32] conducted a systematic literature review and identified more than 50 Long-term effects of COVID-19, with the most common being fatigue, headache, attention disorder, hair loss, and dyspnea. Halpin *et al*[33] identified fatigue, breathlessness, anxiety/depression, concentration problems, and pain among the five most common post-discharge symptoms in 100 patients hospitalized with COVID-19 (ward and ICU). A recent analysis of new ICD-coded outpatient symptoms among non-hospitalized COVID-19 patients 28-108 d post COVID-19 diagnosis lists that long-term symptoms such as throat and chest pain, shortness of breath, headache, malaise, and fatigue are the most common issues experienced by COVID-19 patients even after they have recovered from the initial infection[24]. We identified the most common ten GI and neurologic symptoms as anxiety, depression, dysphagia, headache, nausea and/or vomiting, myalgia, gastroesophageal reflux, fatigue, abdominal pain, and vertigo, highlighting symptoms common to both prior inpatient and outpatient-based studies and raising additional symptoms for consideration. Among the top 50 symptoms identified in our study, some have not been previously reported, or only reported in small series or single case studies. These include patient-level symptoms that may have previously been obscured in diagnoses or groups of symptoms, such as "cutaneous signs" like skin rash or lesion while our study captured individual symptom descriptors such as "erythema", "itching", and "rash". Similarly, our findings of "visual changes" and "abnormal gait"[34] have not been duly reported in most studies that we screened for this review. It is also interesting that in the general symptom category, both weight gain (11256) and weigh loss (1906) were reported in the same and only one paper among the five reviewed. It is therefore possible that obscure and uncommon symptoms are picked up by the questionnaires or survey questions posed by the investigators that are holistically conducting their research as opposed to selecting the most common symptoms to show in the analysis of their collected data.

The main limitation of our review is the small number of published papers that were used in collating the various common symptoms experienced by LC/PASC patients and the limited sources of data available exclusively in the GI and neurological category of symptoms. Based on our descriptive analysis of the symptoms and the varying degree of sample sizes in each cohort study from the five papers, there is a positive correlation between GI and neurological symptoms regardless of the significance level. It is important to point out that contrary to the published reports, fatigue was not a major symptom among those PASC patients experiencing the most GI or neurologic symptoms. Further work is needed in this area to clearly understand why such a discrepancy exists. It is important that future studies bring light and clarity to the underlying causes of the disproportionate infection, duration of symptoms, or mortality in minority populations. There may also be similarities in symptoms, in patients of other countries that could shed light on the long-term effects of COVID-19.



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Figure 4 Gastrointestinal, neurologic, and comorbidities post-acute sequelae of severe acute respiratory syndrome coronavirus 2 symptoms. A: Gastrointestinal post-acute sequelae of severe acute respiratory syndrome coronavirus 2 infection (PASC); B: Neurologic PASC; C: Comorbidities in PASC patients. GI: Gastrointestinal; CAD: Coronary artery disease; CVA: Cerebral vascular accident; COPD: Chronic obstructive pulmonary disease; HIV: Human immunodeficiency virus infection.

CONCLUSION

LC/PASC leads to prolonged and debilitating symptoms beyond 30 d after SARS-CoV-2 infection. Neurological symptoms, such as encephalopathy, myalgia, and anosmia, are common in LC/PASC patients. GI sequelae, including difficulty swallowing and abdominal pain, are frequently observed. Females may be more susceptible to LC/PASC. Anxiety and depression are prevalent, resembling features of PTSD. Fatigue's discrepancy in symptom reporting requires further investigation. More research is needed to understand the long-term effects and potential treatments for LC/PASC

patients.

ARTICLE HIGHLIGHTS

Research background

The respiratory infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for a global epidemic, extends beyond pulmonary issues. It induces multi-organ disorders, affecting cognition (neurological) and gastrointestinal (GI) function. Long-term repercussions of this infection are termed post-acute sequelae of SARS-CoV-2 infection (PASC) or long coronavirus disease 2019 (COVID) (LC). This review aims to analyze current knowledge and outcomes of long-term neurological and GI effects in adult cohorts, encompassing United States minority populations.

Research motivation

This research is motivated by the need to explore and understand the long-term neurological and gastrointestinal effects of COVID-19 in adult cohorts, with a particular focus on United States minority populations. By investigating these long-term sequelae, the study aims to contribute to the existing knowledge, provide insights into potential health impacts, and potentially lead to better management and care for individuals affected by PASC or LC.

Research objectives

To identify and document the neurological symptoms and cognitive impairments experienced by individuals who have recovered from COVID-19, as well as to examine and characterize the gastrointestinal symptoms and disorders observed in post-COVID-19 patients. The research seeks to determine the prevalence and severity of these long-term effects, considering the disproportionate affliction in United States minority populations. Furthermore, the study aims to investigate contributing factors, risk factors, comorbidities, and demographic variables associated with the development of long-term neurological and GI sequelae in post-COVID-19 individuals. We also wish to explore the underlying mechanisms and pathophysiological processes that may lead to these long-term effects. Future investigations might include a comparison with control groups to discern the specific impact of the virus on neurological and GI systems. Moreover, future research will analyze potential disparities in the prevalence and outcomes of long-term effects among different racial and ethnic groups. Ultimately, the study's findings will provide valuable clinical insights and contribute to public health knowledge, offering evidence-based information for improved assessment, management, and care of individuals experiencing long-term neurological and GI effects following COVID-19. The researchers will suggest recommendations for healthcare providers, policymakers, and future researchers to address the challenges posed by LC in diverse populations.

Research methods

PubMed and Google Scholar were searched using relevant terms, and data from five studies were analyzed, comprising 27383 patients with persistent neurological and GI sequelae.

Research results

The study revealed several prominent symptoms, such as anxiety, depression, dysphagia, headache, vomiting, nausea, gastroesophageal reflux, fatigue, and abdominal pain, among LC patients. Notably, individuals with comorbidities and metabolic syndromes faced an increased risk. While most patients were of European American descent, the impact on African American individuals requires more extensive investigation. The underlying reasons for these symptoms remain uncertain, emphasizing the necessity for further research into the long-term effects of the SARS-CoV-2 on diverse populations.

Research conclusions

The study concludes that LC is associated with a range of significant symptoms, including anxiety, depression, dysphagia, headache, vomiting, nausea, gastroesophageal reflux, fatigue, and abdominal pain. Patients with comorbidities and metabolic syndromes are at higher risk of experiencing these long-term effects. The research highlights the need for further investigation into the impact of LC on African American populations and emphasizes the uncertainty surrounding the underlying causes of these symptoms. Overall, the study underscores the importance of understanding the long-term consequences of SARS-CoV-2 infection in different populations.

Research perspectives

The study on LC symptoms reveals the importance of considering research perspectives that can enhance our understanding of the condition's impact on different populations. To achieve this, future studies should prioritize diverse and representative samples, particularly including African American populations and other minority groups. Exploring potential biological, socioeconomic, and cultural factors that influence LC symptoms in diverse communities can provide valuable insights. Additionally, investigations into health disparities and the intersectionality of comorbidities will aid in addressing specific challenges faced by various racial and ethnic groups. Culturally tailored interventions, improved healthcare access, and preventive measures are crucial for managing LC and ensuring better long-term outcomes for affected individuals from diverse backgrounds.

FOOTNOTES

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Country/Territory of origin: United States

ORCID number: Zaki A Sherif 0000-0002-1772-7041; Hassan Brim 0000-0001-7055-2298; Hassan Ashktorab 0000-0002-4048-4666.

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SCIENTOMETRICS

Physician-scientists or celebrities? Kardashian-index of gastroenterologists

Onyinye Ugonabo, Saad Ullah Malik, Usman Ali Akbar, Zarlakhta Zamani, Wesam Frandah

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Onyinye Ugonabo, Department of Internal Medicine, Marshall University Joan C. Edwards School of Medicine, Huntington, WV 25701, United States

Saad Ullah Malik, Department of Cardiology, Geisinger Medical Center, Danville, PA 17821, United States

Usman Ali Akbar, Department of Internal Medicine, West Virginia University-Camden Clark Medical Center, Parkersburg, WV 26101, United States

Zarlakhta Zamani, Department of Internal Medicine, Centinela Hospital Medical Center, Inglewood, CA 90301, United States

Wesam Frandah, Department of Gastroenterology, Marshall University Joan C. Edwards School of Medicine, Huntington, WV 25701, United States

Corresponding author: Onyinye Ugonabo, MD, Doctor, Department of Internal Medicine, Marshall University Joan C. Edwards School of Medicine, 1249 15th St, Huntington, WV 25701, United States. onyinyeugonabo99x@gmail.com

Abstract

BACKGROUND

The coronavirus disease 2019 pandemic unleashed a flood of untrustworthy information on social media platforms, resulting in the unfortunate consequence of expert scientists' opinions getting lost amidst the chaotic sea of misinformation. The question of how much influence these esteemed scientists hold on social media platforms remains elusive. To address this scientific quandary, we sought to explore the concept of the Kardashian index (K-index), a term introduced by Hall in 2014. This metric provides a rudimentary means of evaluating whether a physician scientist's popularity on social media aligns with their significant scientific contributions.

AIM

To evaluate if a Gastroenterologist physician's popularity on social media is at par with their scientific contributions (research articles and publications).

METHODS

We conducted an extensive search to identify all gastroenterologists actively practicing and associated with the top 100 hospitals as reported by the United States News. We collected specific data on a sub-group including their names,

affiliations, degrees, and sub-specializations. To gauge their social media popularity, we utilized the K-index calculation which is determined by dividing the actual number of Twitter followers by the number of researcher's citations. The expected number of followers (F) is calculated using the formula F = 43.3 C ^ 0.32, where C represents the number of citations.

RESULTS

Physicians affiliated with the Mayo Clinic emerged as the most prominent presence on Twitter, constituting 16% of the total. They were followed closely by physicians from Mount Sinai Hospital (9%) and the University of Michigan Hospital (9%). Surprisingly, 76% of the physicians evaluated exhibited a low K-index, falling within the range of 0 to less than 2. This suggests that a significant number of highly influential physician-scientists are not receiving due recognition, as indicated by their relatively low number of followers. On the other hand, 24% of the physicians had an inflated K-index, exceeding 5, which positioned them as the "Kardashians". These individuals enjoyed greater social media popularity than their actual scientific contributions. Interestingly, our analysis revealed no discernible association between sex and K-index (*P* value of 0.92).

CONCLUSION

In the gastroenterology field, our study estimated that a majority (76%) of highly researched physicians are undervalued despite their significant scientific contributions.

Key Words: Kardashian index; Gastroenterology Twitter; Kardashian index of gastroenterology; Physician-scientists; Social media; Physician celebrities

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Core Tip: Twitter has become the most used social media by physicians to connect with colleagues and disseminate health information. People are prone to believing any information posted on social media to varying degrees. Thus, when do we consider this information factual and the truth? In our study, we used the Kardashian index to estimate whether the Twitter followers of the top 100 gastroenterologists are relatable to their scientific contributions in terms of citation of their scholarly works, and we found the majority of the gastroenterology physician scientists scored < 2 because of inactivity on Twitter and very low number of followers. We postulated that an avenue to mitigate the prevalence of misinformation on social media could emerge by involving a greater number of physician-scientists on this platform. Their engagement, coupled with the dissemination of their research discoveries could contribute significantly to this endeavour.

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INTRODUCTION

The role of social media platforms in the promotion and exposure of medical literature has shown marked improvement in the last several years. Numerous societies and journals patronize Twitter, Facebook, and other platforms to bring their readership closer to their published articles. Twitter, with an estimated 300 million monthly users, has become a major nerve center between medical practitioners, patients, journals, and healthcare for discussion and transit of information. A directory of researchers on Twitter does not exist, hence it is difficult to estimate the percentage of researchers that patronize this popular media[1] A systematic review of scholars on Twitter by Costas *et al*[2] identified > 385000 scholars linked to a Twitter account, however, the number varies based on level of productivity, identifying scholars with a higher level of productivity to have a stronger presence on Twitter especially those in social science and humanities by discipline. The K-index was proposed by Neil Hall in 2014 to study the effect the number of Twitter followers a physician or scientist has on the degree of citation of their research publications[3]. It was inspired by a popular celebrity with millions of followers on social media, Kim Kardashian. Her status, though not related to academics galvanized a new index to measure the dissemination of research to reach millions of people. The concept of the K-index centers around the fact that the popularity of a clinician on Twitter depends on the number of followers. The more followers, the more fame and success which in turn increases a gastroenterologist's research visibility, thus increasing the likelihood of having more citations of research articles. A couple of studies have been published for other specialties correlating the effect of their social media influence on their scientific works. We, therefore, sought to evaluate that of gastroenterologists. Our study hypothesized that many high-impact gastroenterology (GI) physicians don't have a significant social media presence limiting their outreach and popularity among the public, despite significant scholarly work.

MATERIALS AND METHODS

We conducted an extensive search using usnews.com to identify all gastroenterologists currently practicing and affiliated with the top 100 hospitals as reported by the United States Health News and World Report. From this comprehensive search, we collected specific sub-group data including names, affiliations, degrees, and sub-specializations. Additionally, we collected other variables such as sex, title [Doctor of Medicine degree (MD) or Doctor of Osteopathic Medicine (DO)], and number of years in practice. We randomized the data using randomization software to ensure unbiased selection and selected the top 2000 gastroenterologists for our study.

Utilizing Twitter as our chosen social media platform, we evaluated the number of followers for each of these selected gastroenterologists, excluding those whose accounts were no longer active. It's important to note that we were unable to determine the occupation of each follower, thus we could not ascertain whether the majority were medical professionals or not.

To authenticate the number of citations, we utilized various publications and citation search engines, including Semantic Scholar, Scopus, ResearchGate, and Google Scholar. The K-index was then calculated using the formula K-index = $F_{(a)}/F_{(c)}$, where $F_{(a)}$ represents the number of Twitter followers and $F_{(c)}$ represents the number of followers based on citations. The actual trend of Twitter follows was described using the formula $F = 43.3\ C^{0.32}$, where F denotes the number of Twitter followers and C represents the number of citations.

A higher K-index suggests a lower proportion of actual scientific contribution in relation to the number of followers on Twitter. Demographic data were presented as mean or median values, and associations were assessed using appropriate statistical tests such as *t*-tests, chi-square tests, and Spearman tests. Further evaluation of significant results was performed using ordered logistic regression. All statistical analyses were conducted using STATA version 15.1.

RESULTS

A total of 1979 GI physicians had analyzable data. 98.8% (n = 1956) were MDs and 1.16% (n = 23) were DOs. Only 6.6% (n = 131) had an extra-degree. The mean years as faculty was 19.8 years (SD = 9.4). Only 16.6% (n = 330) had an active Twitter account and among them 66% (n = 218) were males, and 34% (n = 112) were females. Only 2.5% (n = 50) of Twitter-using physicians had an extra degree and their median years as faculty was 18 (SD = 8.6). The most dominant presence on Twitter was by physicians from Mayo Clinic (16%), followed by Mount Sinai Hospital (9%) and the University of Michigan Hospital (9%) (Figure 1, Table 1). Approximately 76% of the physicians studied exhibited a low K-index (ranging from 0 to less than 2), suggesting a significant under-representation of highly influential physician-scientists on Twitter. Conversely, 24% displayed an inflated K-index (> 5), classifying them as "Kardashians" or physician celebrities, implying that their popularity surpasses their actual scientific contributions. (Figures 2 and 3). We found no association between sex and K-index (P value = 0.92). There was a significant association between K-index and extra degree (P = 0.17). A higher K-index was associated with lower odds of an advanced degree [odds ratio (OR): 0.74, 95% confidence intervals (95%CI): 0.57-0.96, P value = 0.03] when adjusted for sex and years as faculty. There was a significant association between the number of years as faculty and K-index (P value = 0.008) but this association did not remain significant when adjusted for sex, years on Twitter, and extra degree (P value = 0.69). A higher number of years of Twitter use leads to higher K-index (OR: 1.39, 95%CI: 1.11-1.74, P value = 0.004) after controlling for sex, years as faculty, and extra degree.

DISCUSSION

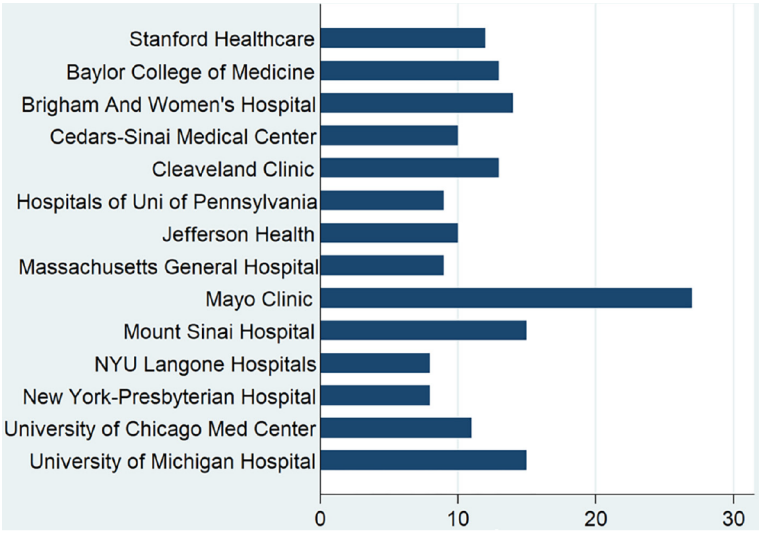
Some gastroenterologists have utilized Twitter as a platform for sharing interdisciplinary discussions and raising awareness about their areas of expertise. Out of the 1979 GI physicians analyzed, only 16.6% maintained an active Twitter account. Among the Twitter users, 76% had a K index ranging from 0 to less than 2 depicting undervalued on social media, despite their scientific contribution. The adoption of social media among physicians significantly increased from 41% in 2010 to approximately 90% in 2011[4]. A study by Madde and Zickuhr[5] suggested that social media usage grew by 43% between June 2009 and 2010, becoming the most time-consuming internet activity[6].

Analysis of social media usage has revealed some notable trends. Twitter appears to be more popular among individuals aged 15 to 29, compared to only around 7% of those over 65-years-old[7]. According to a survey conducted by Woitowich *et al*[8] on sex differences in social media usage among physicians, the majority of respondents were women. However, men were found to utilize social media more extensively for building professional networks and staying updated on research and clinical topics[9]. Another study by Demailly *et al*[10] focused on anesthesia and critical care researchers reported lower visibility of women compared to their male counterparts on a scientific research-dedicated social network called ResearchGate. Similarly, our study found that male GI physicians had a higher prevalence of Twitter usage (66%) compared to females (33%).

Although our data analysis did not show a significant difference between sex and K-index (P value = 0.92), it revealed an association between years of experience as faculty and K-index. However, when adjusting for sex, years on Twitter, and additional degrees, this association no longer remained significant (P value = 0.69). It is important to note that the K-index, used as a measure of celebrity status, has been referenced to evaluate researchers in certain clinical specialties such as cardiology and interventional neuroradiology. The K-index studies conducted by Khan *et al*[11] and Vilanilam *et al*[12] on cardiologists and interventional neuroradiologists, respectively, demonstrated that most physicians on Twitter had a

Table 1 Top 15 hospitals contributing to most gastroenterology physician users on Twitter

	Frequency	Percent
Stanford Healthcare-Stanford Hospital	12	6.90
Baylor College of Medicine	13	7.47
Brigham and Women's Hospital	14	8.05
Cedars-Sinai Medical Center	10	5.75
Cleveland Clinic	13	7.47
Hospitals of University of Pennsylvania-Penn Presbyterian	9	5.17
Jefferson Health-Thomas Jefferson University Hospitals	10	5.75
Massachusetts General Hospital	9	5.17
Mayo Clinic	27	15.52
Mount Sinai Hospital	15	8.62
NYU Langone Hospitals	8	4.60
New York-Presbyterian Hospital-Columbia and Cornell	8	4.60
University of Chicago Medical Center	11	6.32
University of Michigan Hospital-Michigan Medicine	15	8.62



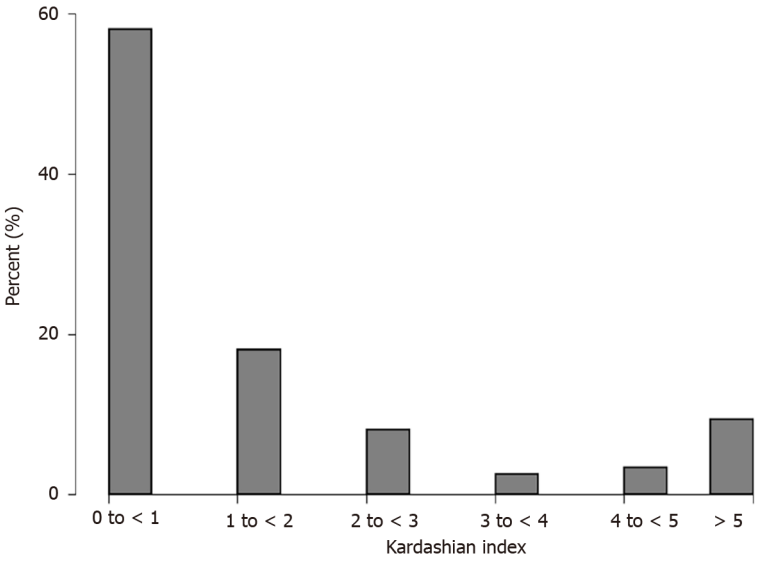
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Figure 1 Top 14 hospitals contributing to most gastroenterology physician users on Twitter.

low K-index between 0 and 2, while only a few were considered the Kardashians or physician-celebrities (K-index > 5), consistent with the findings of our study. However, it should be emphasized that the K-index formula has limited scientific value and may not accurately reflect a physician's true value and worth[3].

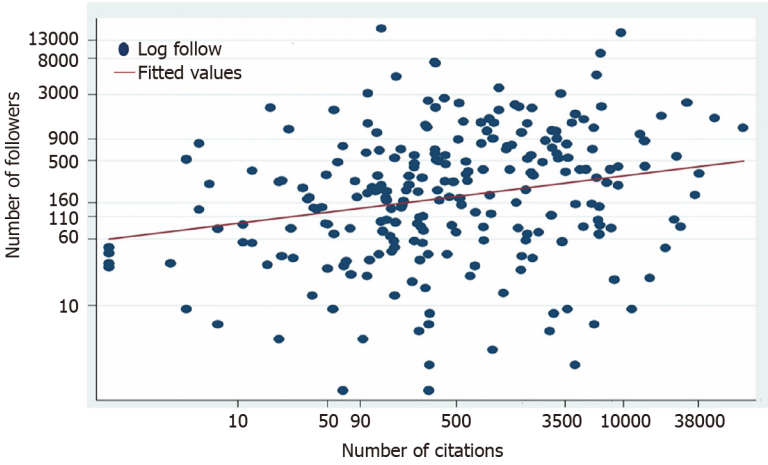
Nonetheless, spending more time on Twitter may have a positive impact on the citation of scholarly works. Recent studies have suggested a positive correlation between highly tweeted articles and increased citations. For instance, a study by Haustein *et al*[13] on tweeting biomedicine, demonstrated a positive correlation between tweeting and citation behavior across various specialties. Eysenbach[14] reported that highly tweeted articles were more likely to be cited than less tweeted articles, with statistically significant Pearson correlation coefficients ranging from 0.42 to 0.72 for log-transformed Google Scholar citations. Gunaratne *et al*[15] illustrated that articles tweeted by authors experienced a 3.08-fold increase in citations within a year and a 1.51-fold increase in total citations. Furthermore, a recent prospective randomized controlled trial study by Luc *et al*[16] found that tweeted articles achieved a significantly higher increase in altmetric scores compared to non-tweeted articles, along with a greater change in citations in 1 year.

The issue of information authenticity on social media is a contentious one, particularly since many individuals rely on social media instead of journals or guidelines. Our hypothesis is that if highly cited GI physicians become more active on Twitter, it may be possible to raise the K-index of a majority of physicians to 3. This would lead to more tweets on published research articles and guidelines, attract more followers, and potentially reduce the dissemination of



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Figure 2 Kardashian index of gastroenterology physicians on Twitter.



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Figure 3 Number of followers of studied gastroenterology physicians on Twitter *vs* the number of citations for their publications.

misinformation. Aside from increasing research citations and providing factual health information to the public, Twitter offers various other benefits. As a GI physician on Twitter, you have the opportunity to network with colleagues, recruit diverse applicants for fellowship programs, participate in discussions like the Monday night inflammatory bowel disease initiated by John Hopkin's GI physician, Dr. Charabaty, and engage in academic forums like Liver Twitter, which focuses on sharing contents related to chronic liver diseases with a global audience[9,17]. GI mentors with high Twitter impact who are advanced in their field can use this medium to promote the accomplishments of their mentees, increase their visibilities, and open up channels for additional career opportunities[18].

Certain limitations are associated with the K-index. Younger researchers tend to score higher due to a faster accumulation of Twitter followers compared to lifetime citations. Additionally, effective communication of scientific knowledge to attract a large audience is a necessary skill. Furthermore, researchers working in fields with highly ranked professionals are more likely to have their articles cited compared to those in smaller fields[19]. Our data analysis revealed that most GI physicians active on Twitter were affiliated with top-ranked hospitals, with Mayo Clinic having the highest percentage (16%) of physicians with an active Twitter presence, followed by Mount Sinai Hospital (9%) and the University of Michigan Hospital (9%).

It is important to acknowledge the limitations of our study. Firstly, our focus was solely on GI physicians on Twitter due to the original Kardashian study's emphasis on Twitter. We were unable to determine how many of the Twitter followers were medical professionals likely to retweet or quote articles. We also were not able to analyze the contents of the Tweets. Although we conducted a thorough search, there remains a chance that certain Twitter accounts might have eluded detection. Lastly, it is worth noting that the Kardashian formula lacks scientific validation to determine a physician's worth. It is common for celebrity physicians to forgo the humorous intent behind the creation of the K-index

by Hall and take it too seriously, misinterpreting it in a different light than it was created. Hall, 2014 advocated that physicians on Twitter may find it useful to calculate their K-index and attach it to their profile.

CONCLUSION

In conclusion, encouraging highly cited GI physicians to be more active on Twitter may have positive implications, including increased citation of research works and the availability of accurate health information. Twitter also offers opportunities for networking, recruitment, and participation in academic discussions. Nonetheless, the K-index has its limitations, and caution should be exercised when interpreting its results. By sharing and retweeting articles, fostering scientific discussions, forming professional connections, and potentially boosting the impact factor of journals through increased citations, Twitter can serve as a valuable platform. However, it is essential to remember that having an opinion on a subject does not automatically make one an expert. Increased participation of GI physician-scientists on Twitter, specifically in sharing new clinical guidelines and research findings, may help combat misinformation within the GI community.

ARTICLE HIGHLIGHTS

Research background

There is a growing recognition among certain physicians about the significance of social media in facilitating the dissemination of research findings. While some physicians are beginning to appreciate the usefulness of social media, a ton of others are yet to comprehend its importance. In 2014, Hall proposed the K-index as a scientific metric aimed at evaluating if a physician's celebrity status on Twitter (assessed by the high number of followers) is at par with their scientific contributions based on the number of cited research work.

Research motivation

The coronavirus disease 2019 pandemic brought about a lot of misinformation on social media relating to its treatment and prevention. Hence, there arose a need to measure the scientific contribution (number of cited research works) of physician celebrities on Twitter. A study on the K-index of cardiology showed that the majority of the cardiologists on Twitter had a K-index < 2 (indicating more research works compared to the number of social media followers). We were motivated to find out the K-index of gastroenterologists on Twitter.

Research objectives

Our objectives are: (1) To assess whether a gastroenterologist's celebrity status on Twitter equates to the number of published and cited research works; (2) to assess the Twitter activity level of the gastroenterologists in the Top 100 hospitals as reported by the United States World News; and (3) to determine the effect of high Twitter followers on the number of cited scholarly works.

Research methods

An extensive search was done to identify all gastroenterologists actively practicing and associated with the top 100 hospitals as the United States News reported. We collected specific data on a sub-group including their names, affiliations, degrees, and sub-specializations. To gauge their social media popularity, we utilized the K-index calculation which is determined by dividing the actual number of Twitter followers by the number of researcher's citations. The expected number of followers is calculated using the formula $F = 43.3\ C^{0.32}$, where C represents the number of citations.

Research results

We found that physicians affiliated with Mayo Clinic emerged as the most prominent presence on Twitter, constituting 16% of the total. They were followed closely by physicians from Mount Sinai Hospital (9%) and the University of Michigan Hospital (9%). 76% of the physicians evaluated exhibited a low K-index of 0-2 which suggested that a significant number of highly influential physician-scientists are not receiving due recognition, as indicated by their relatively low number of followers. However, 24% of the physicians had an inflated K-index of > 5, which positioned them as the "Kardashians" or physician celebrities. These individuals enjoyed greater social media popularity than their actual scientific contributions.

Research conclusions

Encouraging highly cited gastroenterology physicians to be more active on Twitter may have positive implications, including increased citation of research works and the availability of accurate health information and research findings for the public. Twitter also offers opportunities for networking, recruitment, and participation in academic discussions.

Research perspectives

Some physicians, though, may misunderstand the intent of creating the K-index measure. We have been able to determine from the literature review that active presence on Twitter as evidenced by increased tweeting and retweeting

of articles can help boost citation and the H-index of scientists. Physicians who are actively invested in research may find an alternative way to get the result of their research findings to the public and increase their visibility. Mayo Clinic has been at the forefront of utilizing socialmedia in health care, embarking on its journey with podcasting in 2005 and eventually expanding to various social media platforms like Twitter, YouTube, and Facebook. Their objective has been to disseminate the knowledge and expertise of their physicians to a wider audience while also providing a platform for patients to share their stories and experiences. By leveraging these channels, Mayo Clinic has effectively made healthcare information and personal narratives easily accessible to a diverse range of individuals. It is not surprising they ranked number 1 in our study. We hope that other programs will adopt and replicate this approach. In the future, we hope for a scientifically proven index or metric to assess a physician's impact and research influence.

FOOTNOTES

Author contributions: Malik US performed the analysis and wrote both the methods and results; Akbar AU and Zamani Z assisted in data analysis and findings; Ugonabo O performed a comprehensive literature review, wrote the introduction, discussion, and edited and merged all of the author's contributions to fit with the Journal's requirements; Akbar AU assisted in reviewing the manuscript; Frandah W did a final review of the manuscript and made significant contributions before submission to the journal.

Conflict-of-interest statement: None to disclose.

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Country/Territory of origin: United States

ORCID number: Onyinye Ugonabo 0000-0002-5837-5062.

Corresponding Author's Membership in Professional Societies: American College of Gastroenterology; American Association for the Study of Liver Diseases.

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SCIENTOMETRICS

Mapping research trends of transarterial chemoembolization for hepatocellular carcinoma from 2012 to 2021: A bibliometric analysis

Na Zhang, Xiao-Feng He, Xiang-Ke Niu

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Na Zhang, Department of General Practice, Affiliated Hospital of Chengdu University, Chengdu 610081, Sichuan Province, China

Xiao-Feng He, Department of Interventional Radiology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong Province, China

Xiang-Ke Niu, Department of Interventional Radiology, Affiliated Hospital of Chengdu University, Chengdu 610081, Sichuan Province, China

Xiang-Ke Niu, Department of Interventional Radiology, Sichuan Cancer Hospital & Research Institute, School of Medicine, University of Electronic Science and Technology of China, Chengdu 610041, Sichuan Province, China

Corresponding author: Xiang-Ke Niu, MD, PhD, Chief Physician, Professor, Department of Interventional Radiology, Affiliated Hospital of Chengdu University, No. 82 North Second Section of Second Ring Road, Chengdu 610081, Sichuan Province, China. niu19850519@163.com

Abstract

BACKGROUND

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the second leading cause of cancer-related deaths. Transcatheter arterial chemoembolization (TACE) is a therapy where drugs aimed to slow or halt tumor development are injected into the artery supplying for HCC tissues. A comprehensive analysis of all the articles on TACE for HCC can give us a general understanding of the progress in this field and provide guidance for future research.

AIM

To analyze and visualize scientific results and research trends in TACE treatment for HCC.

METHODS

The "Web of Science" database was used to identify articles regarding TACE for the treatment of HCC from 2012 to 2021. VOSviewer and CiteSpace were used to analyze the publications trends, collaboration between countries/institutions/authors, and the co-occurrence of keywords, keyword bursts, and references.

RESULTS
A total of 5728 original articles on TACE for HCC were retrieved. Regarding the volume of publications, the total number of yearly publications showed a generally increasing trend. China had the highest number of articles, while the United States achieved the highest Hirsch index and highest number of citations. The Sun Yat-sen University in China was most prolific institution. The most active author was Park, J.W from South Korea. The Journal of Vascular and Interventional Radiology (234 articles) was the most productive journal. There is a growing trend toward international collaboration in TACE for HCC. Cluster networks of co-cited references suggested that practice guidelines and targeted therapies are an essential theme in this field. In addition, cluster analysis based on keyword co-occurrence identified the research topic "prediction of TACE treatment" as a hotspot, and propensity score matching can be used to help investigators conduct innovative studies in the future.

CONCLUSION
The results of our bibliometric analysis provide the latest trends and hot topics in TACE therapy for HCC.

Key Words: Transarterial chemoembolization; Hepatocellular carcinoma; VOSviewer; CiteSpace; Bibliometrics

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Core Tip: Transarterial chemoembolization (TACE) has been considered the standard of care for intermediate stage hepatocellular carcinoma (HCC). Bibliometric analysis assesses the scientific activity in a given field. Based on the assessment of database and literature characteristics, bibliometrics can estimate developing trends in a scientific manner and expose research frontiers to researchers. Knowledge of the literature in TACE treatment for HCC can lead to a better understanding of the research trends in the field. The results of this bibliometric analysis provided the updated trends and hot topics in TACE treatment of HCC.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the second leading cause of cancer-related deaths[1]. Approximately 70% of patients with HCC are diagnosed at an intermediate to advanced stage, when curative treatment is no longer feasible[2]. Conventional transarterial chemoembolization (cTACE) is performed by injecting chemotherapeutic agents and lipiodol emulsion through a catheter into the tumor supply artery under the guidance of medical imaging, followed by the injection of gelatin sponge particles to embolize the tumor vessel[3]. According to the current guidelines[4,5], TACE is the recommended first-line treatment for patients with Barcelona Clinic Liver Cancer stage B (BCLC-B) disease, defined as a multinodal tumor burden, preserved liver function and good performance status.

Recent advances in TACE techniques, such as the well-known drug-eluting bead TACE (DEB-TACE), have demonstrated the potential to improve the overall survival of patients with intermediate to advanced HCC[6,7]. Studies on the combination of TACE with other treatment modalities, such as thermal ablation[8], radiotherapy[9] or systemic therapy[10], have been actively conducted and have achieved encouraging results. A comprehensive analysis of all the articles on TACE for HCC can give us a general understanding of the progress in this field and provide guidance for future research.

Bibliometric analysis assesses the scientific activity in a given field[11]. Based on the assessment of database and literature characteristics, bibliometrics can estimate developing trends in a scientific manner and expose research frontiers to researchers[12]. In recent decades, a large number of bibliometric analyses have been utilized in the medical field[13]. However, comprehensive bibliometric studies on TACE for HCC are still lacking. Therefore, the aim of this study was to systematically analyze studies on TACE for HCC to determine its current status and assess future trends.

MATERIALS AND METHODS

Data sources and search strategies

Ethics Committee approval was not required for this study as it did not involve intervention or data collection in animal or clinical trials. Articles were obtained from the Web of Science (WoS) with a time limit of January 1, 2012 to December 30, 2021, using the following search terms: TS = (Hepatocellular Carcinoma OR HCC OR Liver Cancer OR Liver

Neoplasms OR Hepatic Neoplasms OR Cancer of Liver) AND TS = (Chemoembolization OR Chemoembolisation OR Transcatheter Embolization OR Transcatheter Chemoembolization OR TACE OR Transarterial chemoembolization). Inclusion criteria included: articles from peer-review journals; written in English. We also excluded reports, books or book chapters, conference proceedings, dissertations, theses, expert opinion, commentaries, editorials, and letters. We excluded 102 studies from the list after removing duplicates because they failed all inclusion criteria or met at least one exclusion criterion. The complete screening process is shown in Table 1.

Data collection

Two reviewers selected the articles independently, and any discrepancy was solved through discussion. We used the bibliometric method, VOSviewer 1.6.13 and CiteSpace 5.8.R2 to identify the data, including authors, affiliations, countries/regions, journals, number of papers and citations, year of publication, Hirsch index (H index), keywords, and references. The H index is used to quantify an individual's scientific research output and measure the impact of his or her research[14]. In this study, journal impact factors (IFs) were collected from journal citation reports for 2021.

Data analysis

In this study, the following network graphs were constructed and visualized using VOSviewer: A network graph of co-cited authors and journals, and a co-occurrence analysis of keywords. According to the frequency of items appearing together, co-citation and co-occurrence analysis can reflect the relationship of the items[15]. Additionally, in the network visualization, each node represents a different item, such as author, journal, country/region, or keyword, and the different colors of the nodes indicate different classification criteria or frequency of occurrence. The size of the node represents the number of citations or occurrences, and larger nodes represent higher-level citations or occurrences[16]. The links between nodes demonstrate the relevance of the items' co-citation or co-occurrence, and the thickness of the lines represents the strength of the links.

In addition, another bibliometric software called CiteSpace is used to show new trends and developments in the scientific literature. It is multidimensional, time-shared, dynamic visual analysis software. Burst detection is used to detect abrupt changes in nodes (including authors, countries, keywords, *etc.*). In this study, we use CiteSpace 5.8.R2 to identify highly cited references and keywords with the strongest citation burst during a certain period.

RESULTS

An overview of publications

From 2012 to 2021, a total of 5728 articles from the last decade were retrieved from WoS, including 72,403 citations for the retrieved articles, with an average number of citations (Nc) per article of 20.67. The H-index for all publications was 111. Supplementary Figure 1 shows the geographical distribution of the total number of papers on TACE for HCC.

Annual trend of paper publication quantity

Figure 1 demonstrates the annual number of papers (Np) related to TACE for HCC. Overall, despite fluctuations over the decade, the number of annual papers increased from 440 in 2012 to 733 in 2021, while Np increased to a peak in 2021.

Analysis of the active countries, institutions and co-authors

The included studies were published in 77 countries and regions in the last decade. We ranked the 10 high-output countries for all authors according to Np (Table 2). China had the highest number of publications (2109), followed by the United States (USA) (1100) and Japan (746). According to the citation analysis, the USA had 31045 citations, followed by China (22453) and Japan (10739). In addition, the USA achieved the highest H-index (71), followed by China (61) and South Korea (49). Overall, 201 institutions contributed to this area. Table 3 displays the five most productive institutions, all of them from Asian countries. Sun Yat-sen University (from China) was the most productive institution, followed by Fudan University (from China) and Seoul National University (from South Korea). Figure 2A shows the trend of global collaboration. There is extensive collaboration among active countries. China has the closest cooperation with the USA and the United Kingdom. Three countries– China, the USA and the United Kingdom–have shown more active cooperation with each other. Figure 2B shows that the organizations are also interacting very closely. Sun Yat-sen University (China) and Johns Hopkins University (USA) are the principal institutions leading collaborative research. The co-author network knowledge map provides relevant links between authors to help researchers access potential collaboration opportunities. As shown in Figure 3, the top three prominent nodes include llovet, J. M., Lencioni R. and Kudo M.

Analysis of highly cited articles

Figure 4 Lists the number of the highest local citation score (LCS) per year for the top 20 articles. The paper is written by Park, J.W in 2015 had the highest LCS of 465 and was ranked first. This is a large multicenter, multinational collaborative retrospective study published in Liver International. The study concluded that TACE was the commonly usedin North America, Europe, China and Korea, regardless of disease stage. Most of the articles with high LCS were conducted in multicenter and international studies and focused on major issues in TACE for HCC. Supplementary Table 1 shows the study characteristics of the top 10 highly cited articles. When the threshold is set at 3, most studies focused on the clinical perspective and conventional TACE-based combination therapies.

Table 1 Topic search quires and refinement procedure

Step	Results	Refinement
1	13393	TS = (Hepatocellular Carcinoma OR HCC OR Liver Cancer OR Liver Neoplasms OR Hepatic Neoplasms OR Cancer of Liver) AND TS = (Chemoembolization OR Chemoembolisation OR Transcatheter Embolization OR Transcatheter Chemoembolization OR TACE OR Transarterial chemoembolization)
2	8530	Refined by publication years: (2012 OR 2013 OR 2014 OR 2015 OR 2016 OR 2017 OR 2018 OR 2019 OR 2020 OR 2021)
3	5830	Refined by document types: Articles
4	5728	Refined by languages: English

TS: Topic; HCC: Hepatocellular carcinoma; TACE: Transarterial chemoembolization.

Table 2 The top ten countries/regions with the highest productivity

Rank	Country/Region	Np	Nc	Average per item	H-index
1	China	2109	22453	13.17	61
2	United States	1100	31045	30.41	71
3	Japan	746	10739	16.03	46
4	South Korea	595	10605	19.92	49
5	Germany	371	5529	16.04	37
6	Italy	279	9252	34.27	45
7	China Taiwan	244	5330	22.84	34
8	France	190	6609	35.97	35
9	Egypt	120	969	8.72	18
10	England	106	3554	34.74	29

H-index: Hirsch index; Np: Number of papers; Nc: Number of citations.

Table 3 The top five most productive affiliations

Rank	Affiliations	Country	Np	Nc	Average per item	H-index
1	Sun Yat-senUniversity	China	228	4360	20.53	34
2	Fudan University	China	146	2991	21.15	27
3	Seoul national University	South korea	112	1497	14.23	23
4	Ulsan University	South korea	111	2033	19.51	25
5	Yonsei University	South korea	109	2126	20.35	25

H-index: Hirsch index; Np: Number of papers; Nc: Number of citations.

Productive journals

A total of 1247 journals published articles in the field. Furthermore, we list the 10 most productive journals with their impact factors in Table 4, and provide a map of the most productive journal in Figure 5. According to statistics from the WoS, Journal of Vascular and Interventional Radiology ranked first with 234 publications in the last decade. Cardiovascular and Interventional Radiology ranks second (220 publications), followed by World Journal of Gastroenterology (143 publications), Medicine (118 publications), and PLoS One (113 publications).

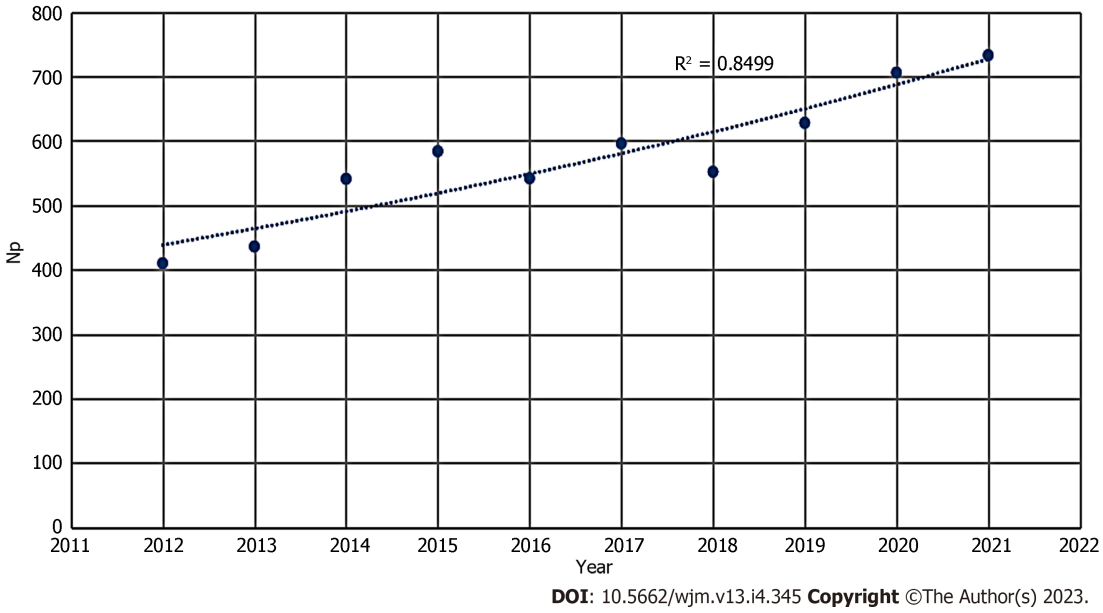
Analysis of co-cited references

Unlike local citation analysis, co-citation networks emphasize research topics that are closely linked to a specific field. The reference co-citation relationship changes over time, and its study can explore the development and evolutionary dynamics of a particular study. Figure 6A shows the mapping of co-cited references based on TACE treatment. Figure 6B depicts the top 20 references with the strongest citation explosion. The most cited references and the highest concen-

Table 4 The top 10 most active journals

Rank	Journal	Np	Nc	Average per item	H-index	IF (2021)
1	*Journal of Vascular and Interventional Radiology*	234	3858	17.41	33	3.682
2	*Cardiovascular and Interventional Radiology*	220	2845	13.86	26	2.797
3	*World Journal of Gastroenterology*	143	3423	24.25	33	5.374
4	*Medicine*	118	1057	9.16	18	1.817
5	*PLoS One*	113	2108	18.94	26	3.752
6	*European Radiology*	108	1441	14.13	21	7.034
7	*Hepatology Research*	81	1434	18.32	21	4.942
8	*Frontiers in Oncology*	77	278	3.83	9	5.738
9	*Oncotarget*	75	1119	15.17	19	/
10	*BMC Cancer*	74	1487	20.18	23	4.638

H-index: Hirsch index; Np: Number of papers; Nc: Number of citations; IF: Impact factors.



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Figure 1 Curve fitting of publications' overall yearly growth trend. Np: Number of papers.

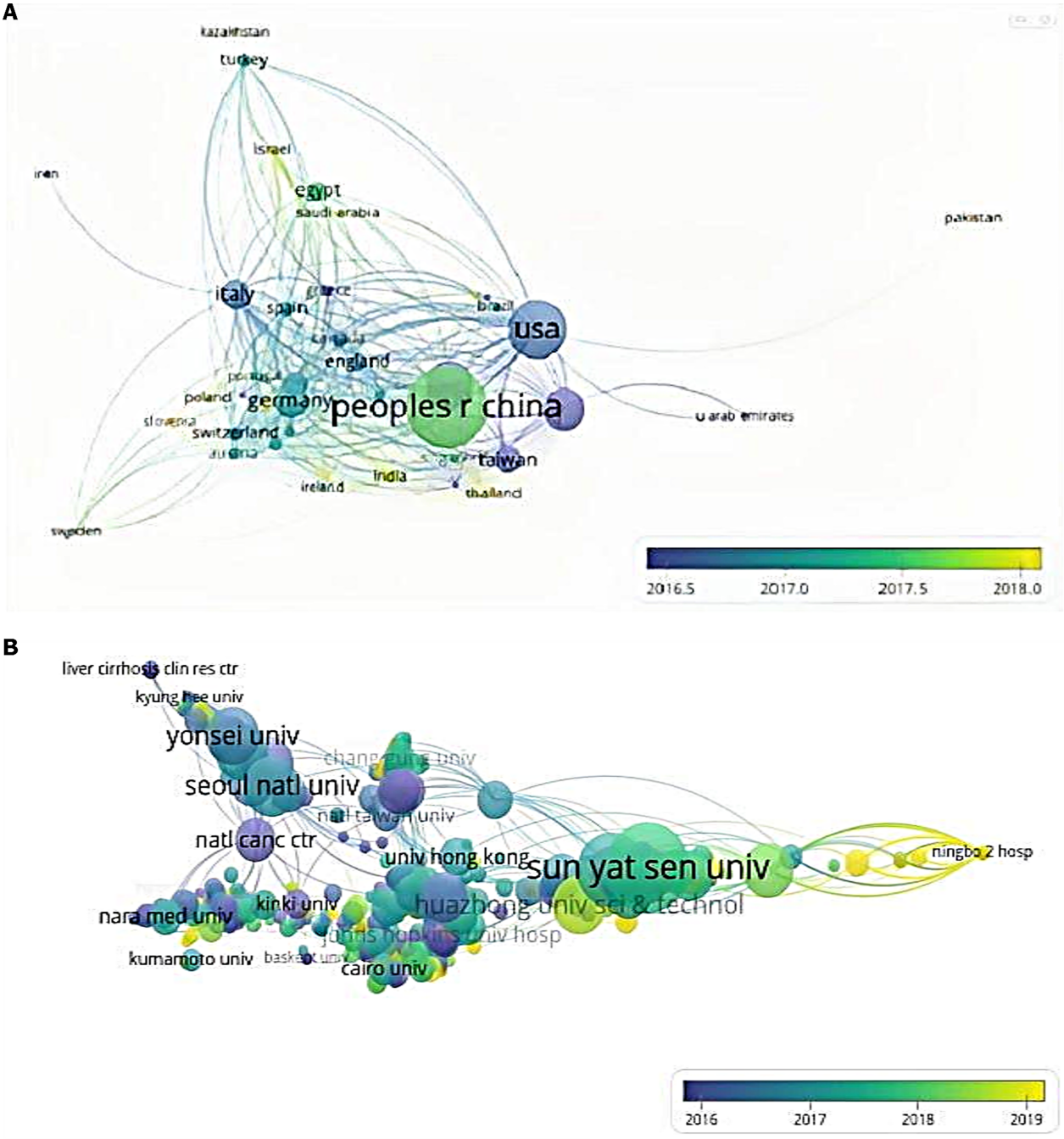
tration of TACE treatment for HCC are "practice guidelines" and "targeted therapy".

Keywords co-occurrence analysis

The high frequency and centrality of keywords allows exploration of core issues and research hotspots of broad interest in the field. A total of 192 terms that appeared more than 20 times were grouped into five clusters. The top three clusters are as follows: Cluster 1 (red) mainly represented the prediction of TACE treatment, as shown in Supplementary Figure 2A. Cluster 2 (green) is focused on vascular endothelial growth factor (VEGF) expression and tumor angiogenesis. Cluster 3 (blue) was the drug-delivery method. The top frequent occurrences of keywords were "HCC", "survival", "management" and "prognosis", indicating that the main focus of research is placed on the prognosis of TACE treatment. Additionally, "drug-delivery method", "targeted therapy" and "response assessment" have long been a focus of research in the field, as shown in Supplementary Figure 2B. Additionally, we found that the propensity score matching was increasingly used to assess TACE safety/efficiency (Supplementary Figure 2C).

DISCUSSION

To the best of our knowledge, this is not the first but comprehensive bibliometric analysis of TACE management in HCC.

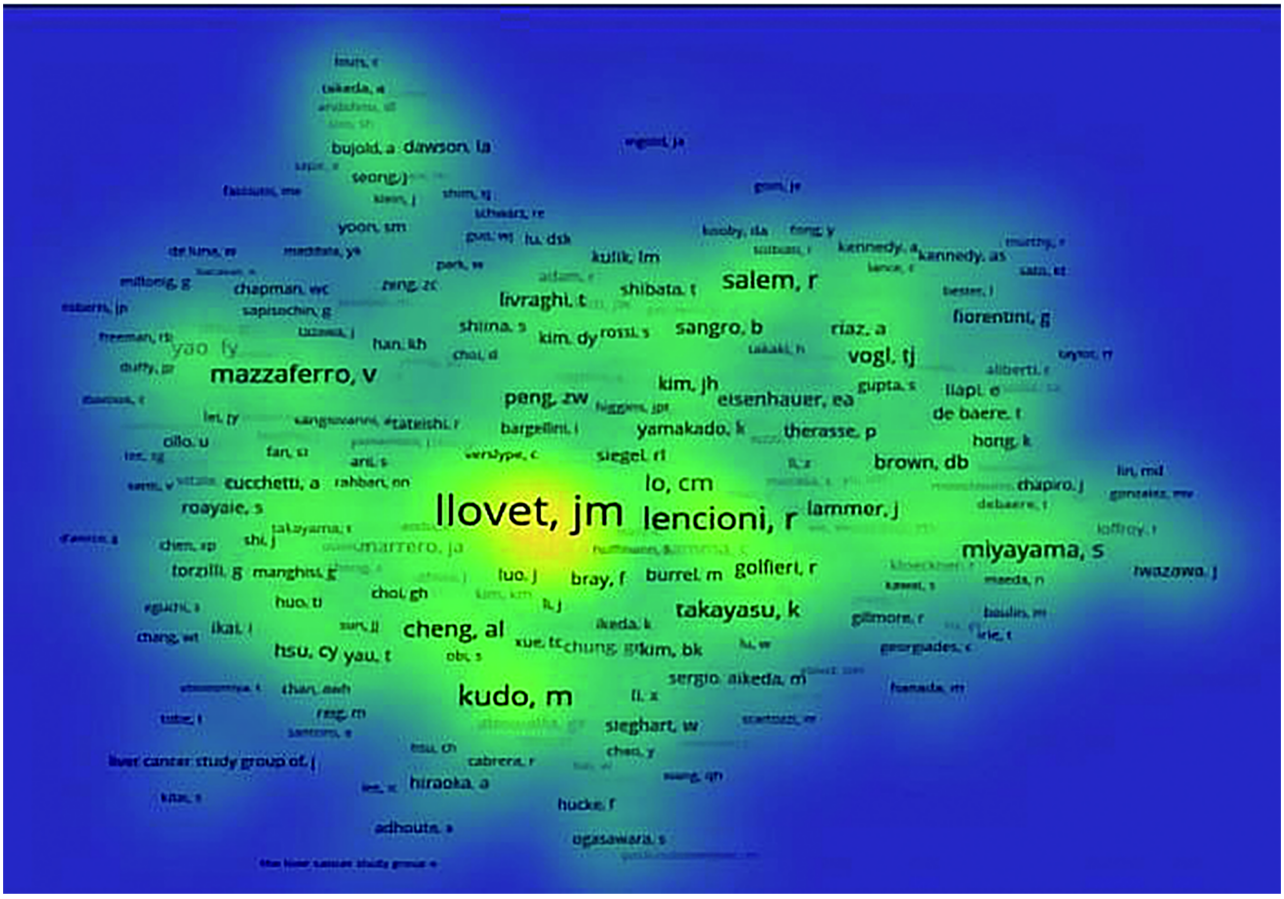


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Figure 2 Shows the trend of global collaboration, the organizations are also interacting very closely. A: Mapping of the main research countries in transarterial chemoembolization (TACE) for hepatocellular carcinoma (HCC). The minimum number of documents of a country was set at 5. Of the 80 countries that were involved in this field, 42 countries met the threshold; B: Mapping of the collaboration network of the main research organizations in TACE for HCC. The minimum number of documents of an organization was set at 15. Of the 2146 organizations that were involved in this field, 74 organizations met the threshold.

A unique database was created, duplicate articles were removed and then selected articles were analyzed along different dimensions: Bibliometrics, demographics, authors, and research trends. The overall increasing trend in the number of papers from 2011 to 2021 reflects a relatively promising research future for TACE in the coming years.

Among the most prolific countries/regions, China ranked first in Np, indicating that China is a highly productive country in this field. Two Chinese institutions and three South Korean institutions came in the top 5 affiliations in Np. According to the GLOBOCAN database, Asia accounts for nearly 72.5% of newly diagnosed cases and 72.4% of deaths from HCC[17]. The BRIDGE study in 2015 concluded that the survival times in South Korea and China were significantly less than that in Japan, which to some extent suggests that these two countries have invested significant resources in recent years. However, compared with China, the USA had a relatively high Nc and H-index. This is because the USA is undoubtedly the most impactful in scientific research fields. The USA has been at the center of academic publishing and has been supported by significant investments in academic research and technological innovation. In recent years, China has also invested heavily in innovative embolic materials for TACE. CalliSpheres® Beads are the first drug eluting beads



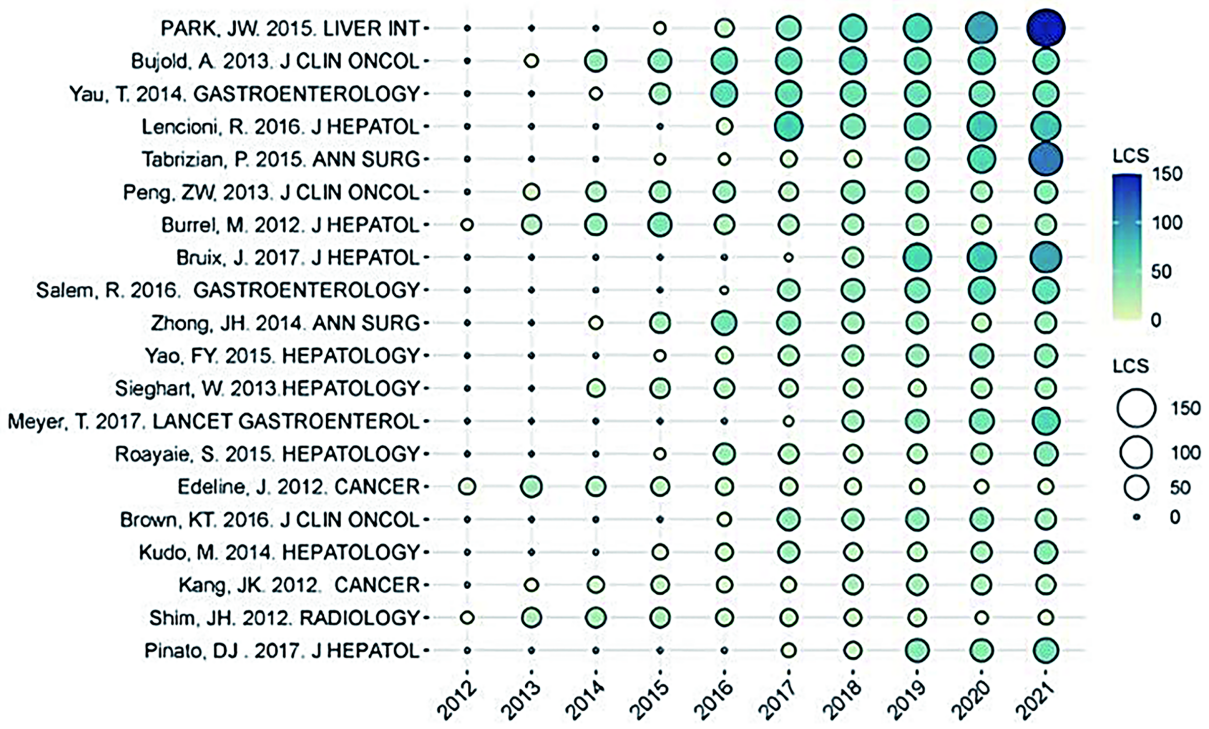
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Figure 3 Co-authorship network of productive authors conducting transarterial chemoembolization research. The minimum number of documents of an author was set at 5. Of the 8975 authors that were involved in the research field, 73 authors met the threshold. Yellow means appearing more frequently, while green means appearing less frequently.

(DEB) product available for HCC treatment in China, and studies have shown that HCC patients treated with CalliSpheres DEB-TACE have a better treatment response and safety than those treated with cTACE[18,19]. Notably, according to the global network of major research institutions and national collaboration, China could have a major impact on research in this area, and despite being a developing country, it has the strongest global collaboration. Large co-authorship nodes in the network diagram show llovet, J. M. occupying the central area, which is not surprising, as he has made significant contributions to this research. In terms of scientific influence a given field, co-authorship and researchers with a high H-index should be comparable, which helps researchers to understand the most influential authors and to seek potential collaboration opportunities with them.

Regarding the top 10 most productive journals, 7 journals have a relatively high IF score of more than 3. This shows that it is not hard to publish studies on TACE in high-quality journals. All of the top 10 high LCS articles were published in these journals with high IFs (IF > 5), including top journals in oncology and liver disease research, such as the Journal of Clinical Oncology and Hepatology. This implies that these journals have published potentially groundbreaking results in the field, which reminds researchers interested in the topic to pay more attention to these journals. We further analyzed the characteristics of the top 10 highly cited articles. In summary, these studies introduce the positive results of recent randomized clinical trials, and describe targeted therapy combination regimens for patients with intermediate stage HCC. Futhermore, we found that all of the studies were clinical studies, suggesting that the researchers in this field have a strong focus on the clinical perspective; however, basic/experimental research leading to major breakthroughs may have a good chance of being published in the top journals in the future.

Co-cited references show that the hotspots that have been of common interest are "practice guideline" and "targeted therapy". TACE is the only guideline-recommended standard of care for intermediate-stage HCC worldwide[20]. Therefore, relevant clinical guidelines are expected to be released every few years in both Western and Asian countries. Researchers have paid more attention to these clinical guidelines when conducting their studies. The explanation is that it is more standardized to conduct studies under clinical guidelines and that the guidelines provide us with possible next research hotspots. For example, the European Association for the Study of the Liver (EASL)-2018 clinical practice guideline sets the goal of treatment for HCC: Linking molecular subclasses in clinical trials to predict the treatment response and overall survival. Targeted therapy [tyrosine kinase inhibitor (TKI)] has been the main treatment for patients with advanced stage HCC for more than 10 years[21]. However, in 2019, lenvatinib was approved as an alternative to sorafenib for first-line targeted therapy for HCC[22]. Currently, studies have mainly focused on the molecular



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Figure 4 The yearly number of local citations of papers with high local citations. The size and colors of the circle represent the high local citations of papers. LCS: High local citations.

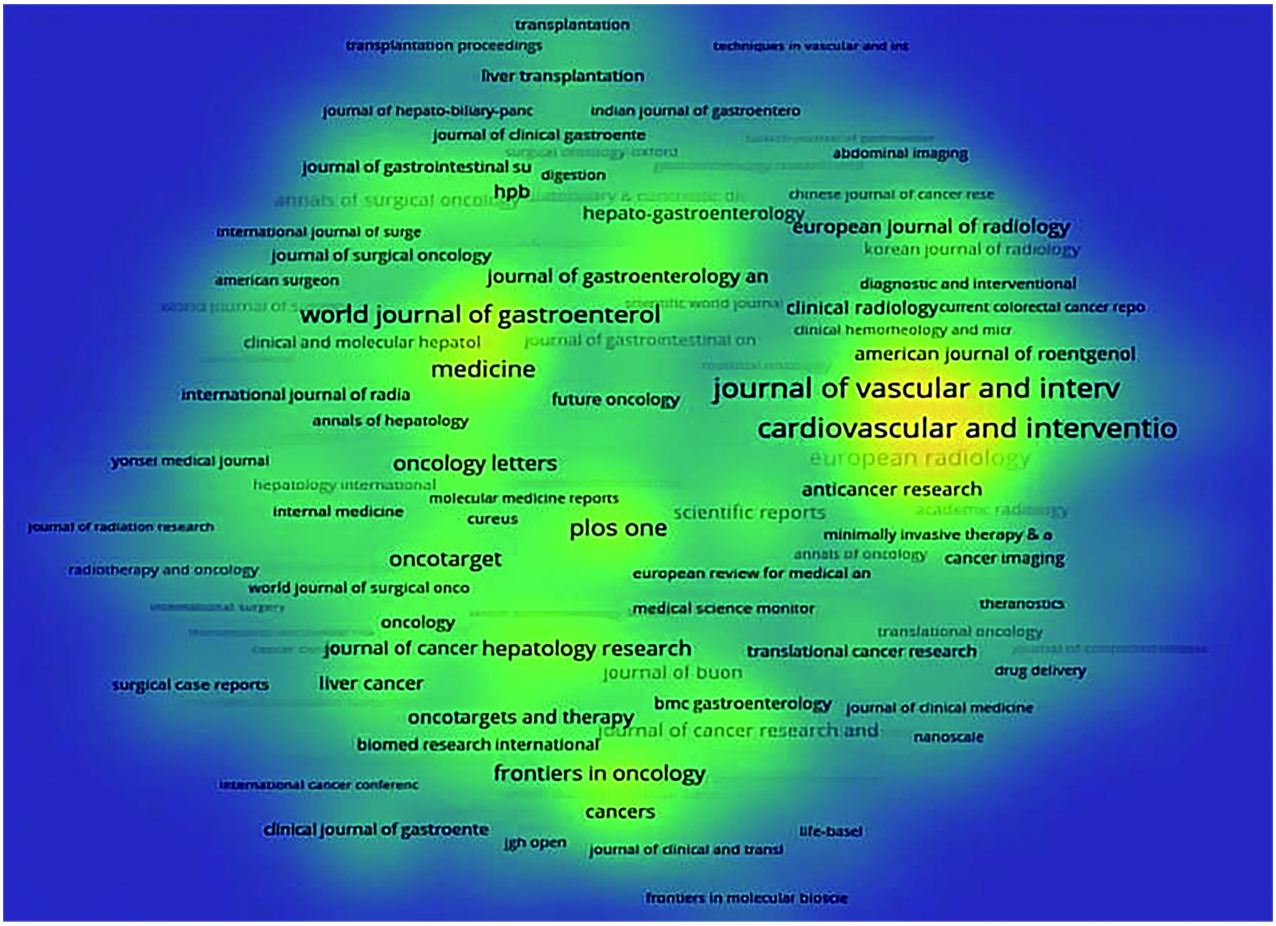
mechanisms responsible for tumor progression and treatment resistance, the sequences and timing of drug administration, and which TKI drugs are most beneficial to patient survival from combination therapy[23,24].

Some emerging research on TACE is gradually becoming a research topic. Cluster 1 (red) primarily represented the prediction of TACE treatment. TACE benefits patients survival; however, repeated TACE therapy progressively impairs liver function. Careful evaluation of the risks and benefits of repeated TACE is needed to improve the long-term outcomes of TACE for HCC. Appropriate decision support has been established during TACE treatment, including the HAP (hepatocellular artery embolization prognosis) score and its modifications (mHAP-II and -III)[25]. A recent study by Wang *et al*[26] developed an easy-to-use model, referred to as the "six-and twelve" score, which stratifies ideal TACE candidates based on the sum of tumor size and number of lesions. Unfortunately, the results of some external validation studies suggested that the model only has a modest predictive ability[27,28]. More recently, Han *et al*[29] developed a survival prediction model that assesses patients prior to initial TACE. Although good results were obtained in the preliminary study, the utility of this model needs to be evaluated in future studies. Novel artificial intelligence approaches based on imaging data clearly hold great promise for the future. A study by Niu *et al*[30] demonstrated the feasibility of a CT-based radiomics model to predict the outcome of sequential TACE treatment.

Cluster 2 is involved in VEGF expression and angiogenesis in HCC. It has been demonstrated that both tumor progression and metastasis are closely related to tumor neoangiogenesis. VEGF is one of the most important angiogenic factors and is highly expressed in HCC tumors; it is associated with tumor angiogenesis, growth, recurrence, and metastasis[31]. Thus, studies on VEGF expression and angiogenesis may lead to novel therapeutic targets for HCC, particularly, in combination with TACE for HCC. Unfortunately, the ideal candidate for molecularly targeted drugs in combination with TACE remains uncertain. Currently, there are more than 1000 clinical trials enrolling patients, demonstrating a dedication to finding novel effective systemic therapeutics or targeting immune checkpoints for the treatment of HCC[32].

In terms of the "delivery method", conventional TACE using iodine oil loaded with anticancer drugs is less effective, and although DEB, including HepaSphere, CalliSpheres and TANDEM, are used, there are still problems of low response rates, as well as chemotherapeutic drug escape and tumor resistance[33]. Currently, the combination of nanodelivery systems with TACE is of interest. Due to the method of medication, the targeting effect of TKIs is poor, while nanodelivery systems can provide an opportunity to overcome the drawbacks and achieve good results[34]. Ding *et al*[35] developed peptide nanogels with a stimulatory response for the delivery of lenvatinib with good tumor suppression and few side effects. Although some nanoparticles (NPs) have been approved by the FDA for clinical use, the safety of NPs has been an unavoidable issue during human applications. Therefore, more in-depth experimental studies and clinical trials on various combinations of TACE and nanospheres are needed to explore the correlation between NP size and optimal drug loading and their impact on drug delivery properties.

In the last decade, technical improvements, mainly include: (1) The treatment should involve a water-in-oil emulsion, which maximize the propensity to target the tumor feeding arteries[36]; (2) improvement of imaging quality during



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Figure 5 Density visualization for the most prolific journals. The minimum number of documents of an organization was set at 5. Of the 857 journals that were involved in this field, 232 journals met the threshold. Yellow means appearing more frequently, while green means appearing less frequently.

fluoroscopy or digital subtracted angiography: Angio Cone-Beam Computed Tomography should be used to detect enhancing tumors, tumor feeders and guide tumor targeting[37,38]; and (3) whenever cTACE is proposed, it should aim at being supraselective-TACE. Regarding the most suitable and appropriate microcatheter for the procedures, 2.4 French (F) was recognized as the upper caliber limit accepted to perform supraselective-TACE. However, all experts agree that a lower microcatheter diameter, 1.5-2.0 F, should be preferred and recommended whenever possible. Recently, balloon occluded TACE (B-TACE), due to its ability to redistribute flow towards lower resistance vascular territories and allowing a pressure-gradient driven embolization, has been shown to improvedrug delivery to target lesion[39,40].The top co-cited reference with the most citation burstiness is "propensity score matching". Propensity score matching (PSM) is one of the most popular statistical methods used to process data from observational studies. In observational studies, there are various bias and confounding variables for some reasons, and the propensity score matching method is designed to reduce the effects of these bias and confounding variables to make a more reasonable comparison between control and experimental groups. PSM has been shown to be comparable to Randomized control trials in producing unbiased estimates of efficacy; however, the cost and research time are significantly reduced[41,42]. A recent study conducted by Marinelli *et al*[43] used propensity score matching and found that TACE can be safely combined with programmed cell death 1 blockers and may lead to HCC downstaging in selected patients.

TACE may also be used as a downstaging therapy prior to hepatectomy or as a bridging therapy prior to liver transplantation in patients with advanced tumors. However, before considering TACE as a treatment option for patients with unresectable HCC, the patient's risk profile, comorbidities, treatment prognosis, and benefits should be taken into account in order to improve overall survival and minimize the occurrence of adverse events. Notably, in addition to the traditional TACE monotherapy, some researchsrs are now focusing on TACE-based combination therapies, such as the combination of TACE and tumor thermal ablation, the combination of TACE and radiotherapy, and the combination of TACE and systemic therapeutic agents. However, further research is needed to determine which combination therapy is more cost-effective in prolonging patient survival.

The present study also has some limitations. First, we only included articles published in English in WoS; therefore, not all publications were considered and the number of citations may have been underestimated. Second, CiteSpace only analyzed the main findings of the studies rather than reviewing the full text; As a result, some important information may have been overlooked. Finally, our results reflect only the current status of TACE for HCC, as data usually change over time.



B

Top 20 References with the Strongest Citation Bursts

References	Year	Strength	Begin	End	2012 - 2021
Lencioni R, 2010, SEMIN LIVER DIS, V30, P52, DOI 10.1055/s-0030-1247132, DOI	2010	89.99	2012	2015	
Llovet J, 2008, NEW ENGL J MED, V359, P378, DOI 10.1056/NEJMoa0708857, DOI	2008	81.96	2012	2013	
Cheng A, 2009, LANCET ONCOL, V10, P25, DOI 10.1016/S1470-2045(08)70285-7, DOI	2009	71.62	2012	2014	
Lammer J, 2010, CARDIOVASC INTER RAD, V33, P41, DOI 10.1007/s00270-009-9711-7, DOI	2010	48.86	2012	2015	
Llovet J, 2008, JNCI-J NATL CANCER I, V100, P698, DOI 10.1093/jnci/djn134, DOI	2008	41.06	2012	2013	
Eisenhauer E, 2009, EUR J CANCER, V45, P228, DOI 10.1016/j.ejca.2008.10.026, DOI	2009	32.85	2012	2014	
Salem R, 2010, GASTROENTEROLOGY, V138, P52, DOI 10.1053/j.gastro.2009.09.006, DOI	2010	31.02	2012	2015	
Bruix J, 2011, HEPATOLOGY, V53, P1020, DOI 10.1002/hep.24199, DOI	2011	128.91	2013	2016	
Jemal A, 2011, CA-CANCER J CLIN, V61, P69, DOI 10.3322/caac.20107, DOI	2011	51.82	2013	2016	
Forner A, 2012, LANCET, V379, P1245, DOI 10.1016/S0140-6736(11)61347-0, DOI	2012	50.18	2014	2017	
European A, 2012, EUR J CANCER, V48, P599, DOI 10.1016/j.ejca.2011.12.021, DOI	2012	108.14	2015	2017	
Torre L, 2015, CA-CANCER J CLIN, V65, P87, DOI 10.3322/caac.21262, DOI	2015	40.56	2016	2019	
Golfieri R, 2014, BRIT J CANCER, V111, P255, DOI 10.1038/bjc.2014.199, DOI	2014	30.04	2016	2019	
Ferlay J, 2015, INT J CANCER, V136, P0, DOI 10.1002/ijc.29210, DOI	2015	35.29	2017	2021	
European A, 2018, J HEPATOL, V69, P182, DOI 10.1016/j.jhep.2018.03.019, DOI	2018	101.94	2019	2021	
Heimbach J, 2018, HEPATOLOGY, V67, P358, DOI 10.1002/hep.29086, DOI	2018	68.85	2019	2021	
Forner A, 2018, LANCET, V391, P1301, DOI 10.1016/S0140-6736(18)30010-2, DOI	2018	54.63	2019	2021	
Kudo M, 2018, LANCET, V391, P1163, DOI 10.1016/S0140-6736(18)30207-1, DOI	2018	52.58	2019	2021	
Omata M, 2017, HEPATOL INT, V11, P317, DOI 10.1007/s12072-017-9799-9, DOI	2017	32.98	2019	2021	
Lencioni R, 2016, HEPATOLOGY, V64, P106, DOI 10.1002/hep.28453, DOI	2016	30.24	2019	2021	

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Figure 6 Shows the mapping of co-cited references based on transarterial chemoembolization treatment, depicts the top 20 references with the strongest citation explosion. A: Density visualization of co-cited references. Of the 68527 references, 1159 (divided into six clusters) were cited at

least 20 times. Yellow means appearing more frequently, while green means appearing less frequently; B: The top 20 co-cited references with the most citation burstiness. Years between "Begin" and "End" represent the period when the reference was more influential. Years in light green mean that the reference has not yet appeared, years in dark green mean that the reference is less influential, and years in red mean that the reference is more influential.

CONCLUSION

In conclusion, the results of our bibliometric analysis provide the latest trends and hot topics in TACE therapy for HCC. Based on the results found by CiteSpace and VOSviewer, we can conclude that current research in this field is focused on survival prediction and TACE-based combined therapies. These results can also help researchers in the field find relevant literature and stay informed about hot topics.

ARTICLE HIGHLIGHTS

Research background

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the second leading cause of cancer-related deaths. Transcatheter arterial chemoembolization (TACE) is a therapy where drugs aimed to slow or halt tumor development are injected into the artery supplying for HCC tissues. A comprehensive analysis of all the articles on TACE for HCC can give us a general understanding of the progress in this field and provide guidance for future research.

Research motivation

The bibliometric analysis can be an important tool to help quantify and evaluate productivity within a scientifc feld, while also providing a comprehensive overview of the literature for students, trainees and experts in addition to identifying potential future research directions.

Research objectives

The aim of this study was to systematically analyze studies on TACE for HCC to determine its current status and assess future trends.

Research methods

The "Web of Science" database was used to identify articles regarding TACE for the treatment of hepatocellular carcinoma (HCC) from 2012 to 2021. VOSviewer and CiteSpace were used to analyze the publications trends, collaboration between countries/institutions/authors, and the co-occurrence of keywords, keyword bursts, and references.

Research results

A total of 5728 original articles on TACE for HCC were retrieved. Regarding the volume of publications, the total number of yearly publications showed a generally increasing trend. China had the highest number of articles, while the United States achieved the highest Hirsch index and highest number of citations. The Sun Yat-sen University in China was most prolific institution. The most active author was Park JW from South Korea. The Journal of Vascular and Interventional Radiology (234 articles) was the most productive journal. There is a growing trend toward international collaboration in TACE for HCC. Cluster networks of co-cited references suggested that practice guidelines and targeted therapies are an essential theme in this field. In addition, cluster analysis based on keyword co-occurrence identified the research topic "prediction of TACE treatment" as a hotspot, and propensity score matching can be used to help investigators conduct innovative studies in the future.

Research conclusions

We found that current research in this field focuses on survival prediction and TACE-based combined therapies. These results can also help researchers in the field find relevant literature and stay informed about hot topics.

Research perspectives

High-quality international multicenter studies are needed to confirm how TACE-based combination therapies better improve patient survival.

FOOTNOTES

Author contributions: Zhang N and He XF contributed equally to this work; Niu XK designed the research study; Zhang N performed the research; He XF contributed analytic tools; Zhang N and Niu XK analyzed the data and wrote the manuscript; all authors have read and approve the final manuscript.

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Country/Territory of origin: China

ORCID number: Na Zhang 0000-0002-3202-3341; Xiao-Feng He 0000-0002-7426-9032; Xiang-Ke Niu 0000-0002-7025-7587.

S-Editor: Liu JH
L-Editor: A
P-Editor: Chen YX

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CASE REPORT

Clinical, imaging, arthroscopic, and histologic features of bilateral anteromedial meniscofemoral ligament: A case report

Juan Bautista Luco, Damian Di Memmo, Valentina Gomez Sicre, Tomas Ignacio Nicolino, Matias Costa-Paz, Juan Astoul, Ignacio Garcia-Mansilla

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Juan Bautista Luco, Tomas Ignacio Nicolino, Matias Costa-Paz, Juan Astoul, Ignacio Garcia-Mansilla, Division of Knee, Department of Orthopaedics, Hospital Italiano de Buenos Aires, Buenos Aires 1181, Argentina

Damian Di Memmo, Department of Radiology, Hospital Italiano de Buenos Aires, Buenos Aires 1181, Argentina

Valentina Gomez Sicre, Department of Pathology, Hospital Italiano de Buenos Aires, Buenos Aires 1181, Argentina

Corresponding author: Ignacio Garcia-Mansilla, MD, Associate Specialist, Division of Knee, Department of Orthopaedics, Hospital Italiano de Buenos Aires, Peron 4190, Buenos Aires 1181, Argentina. ignaciogmansilla@gmail.com

Abstract

BACKGROUND

The anteromedial meniscofemoral ligament (AMMFL) is a very rare entity, commonly unrecognized and underreported. Although it was not proved to be a cause of anterior knee pain, concerns have been raised on the relationship between the presence of this structure and medial meniscus injury secondary to its abnormal motion. Regarding histologic examination, some studies have shown meniscus-like fibrocartilage, while others have identified it as ligament-like collagenous fibrous connective tissue.

CASE SUMMARY

We report the case of a 34-year-old ballerina with an AMMFL associated with a torn medial meniscus of both knees. Surgery was performed to treat the meniscal injury and two biopsies of each AMMFL were taken in different locations to define the histopathological composition. Histologic examination revealed fibrocartilaginous tissue compatible with meniscus. Follow-up evaluation one year after surgery evidenced full remission of symptoms and the patient had resumed her athletic activities.

CONCLUSION

Clinical, magnetic resonance imaging, arthroscopic, and histological features have been carefully described to better characterize the AMMFL.

Key Words: Meniscofemoral; Ligaments; Knee arthroscopy; Histology; Case report

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Core Tip: The anteromedial meniscofemoral ligament (AMMFL) is a very rare entity, commonly unrecognized and underreported. Concerns have been raised on the relation between the presence of this structure and medial meniscus injury. Regarding histologic examination, some studies showed meniscus-like fibrocartilage, while others have identified it as ligament-like collagenous fibrous connective tissue. We report the case of a 34-year-old ballerina with an AMMFL associated with a torn medial meniscus of both knees. Surgery was performed to treat the meniscal injury and two biopsies of each AMMFL were taken in different locations to define the histopathological composition. Clinical, magnetic resonance imaging, arthroscopic, and histological features are carefully described to better characterize the AMMFL.

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INTRODUCTION

Given the interest on meniscal preservation and transplantation, the insertion zones of the meniscus have been widely investigated. Recent anatomical studies have demonstrated several common patterns of attachment of the anterior horn of the medial meniscus (AHMM)[1-4]. According to Berlet and Fowler[3], there are four distinct insertion patterns based on bony landmarks. In type I (59%), the meniscus is inserted in the flat area between the articular surfaces (intercondylar region), providing a very firm attachment. In type II (24%), the meniscus is inserted medially, closer to the articular surface, also providing a strong tibial fixation. Type III insertions (15%) are very anterior and offer little resistance to anterior movement of the meniscus. Finally, type IV (3%) has no solid bony attachment and only the coronary fibers restrain the displacement of the anterior horn of the meniscus. Ohkoshi *et al*[2] subdivided type IV pattern based on the structure to which the AHMM is anchored: Transverse ligament (49%), anterior cruciate ligament (ACL) (38%), coronary ligament (11%), and infrapatellar synovial fold (2%). At last, Cha *et al*[5] subdivided the ACL type into ACL and intercondylar notch (ICN) type. McCormack and McGrath[6] named this ligamentous structure the anteromedial meniscofemoral ligament (AMMFL).

Several studies, mostly case reports, have documented the AMMFL with a wide variation in prevalence (1.2%-15%)[7, 8] and several characteristics of this structure are still not well understood. Although the AMMFL was not proved to be a cause of anterior knee pain, concerns have been raised on the relation between the presence of this structure and medial meniscus injury secondary to its abnormal motion[2,6]. Regarding histologic examination of the AMMFL, some studies showed meniscus-like fibrocartilage, while others have identified it as ligament-like collagenous fibrous connective tissue [8,9].

Our knowledge of the AMMFL is based on very limited data. The aim of our work was therefore to describe the clinical, magnetic resonance imaging (MRI), and arthroscopic findings, as well as the histological composition of the AMMFL, through the case of a bilateral AMMFL in a 34-year-old ballerina.

CASE PRESENTATION

Chief complaints

A 34-year-old ballerina presented to our clinic referring bilateral knee pain of 10 mo long. This pain impaired her performance and provoked symptoms during her daily activities.

History of present illness

A partial meniscectomy in the patient's right knee was performed 8 years ago in another hospital.

History of past illness

The patient denied any other medical history.

Personal and family history

There was no personal and family history.

Physical examination

The patient appeared to be in good physical condition, with a height of 1.68 meters and a body mass index of 23. Examination revealed pain and tenderness over the medial joint line of both knees. The McMurray and Appley tests were positive on the medial side. No restriction in range of motion or laxity was detected.

Laboratory examinations

No laboratory examinations were performed.

Imaging examinations

The MRI of both knees revealed a homogeneous low-signal linear structure on T1- and T2-weighted images, coursing anteriorly to the anterior aspect of the ACL and connecting the medial portion of the AHMM to the posterolateral ICN (Figure 1). This structure was best seen on sagittal view (Figures 1B and F). The MRI of the right knee also showed surgical traces of the previous partial medial meniscectomy together with a tear of the posterior horn of the medial meniscus (Figures 1C and D). The left knee showed a tear of the posterior horn of the medial meniscus (Figures 1G and H).

Further diagnostic work-up

Plain knee and long-leg X-rays did not show bony abnormalities or coronal alignment deformity.

FINAL DIAGNOSIS

Bilateral AMMFL combined with a torn medial meniscus.

TREATMENT

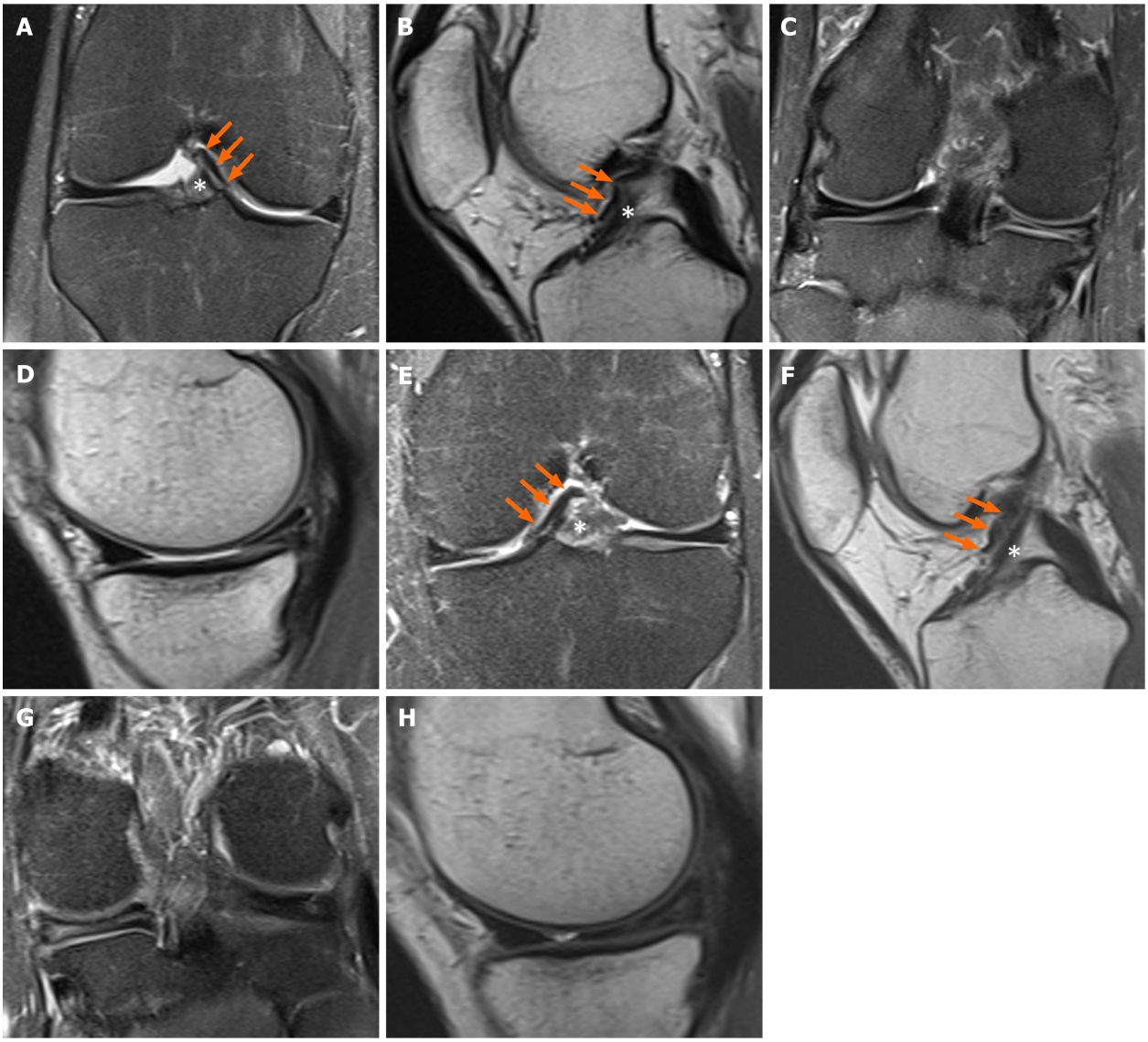
Diagnostic arthroscopy was performed together with a partial meniscectomy of both knees. Figure 2 shows the intraoperative findings of both knees. The AMMFL was visualized on both sides as an anomalous band-shaped structure covering the anterior portion of the ACL (Figures 2A and D). The band was originated at the AHMM and was attached to the posterolateral wall of the ICN. A fine band of synovial tissue engaged the AMMFL with the ACL. In both knees, the AHMM lacked a robust attachment to the tibia. Instead, it was connected to the tibia by meniscocapsular soft tissue. Therefore, the AHMM was noted to be abnormally mobile on palpation. No impingement of the AMMFL with the ICN was observed after mobilizing the knee through full range of motion. Therefore, we did not resect the AMMFL, preserving the structure. Two biopsies were taken from the AMMFL of each knee for histopathological examination, one proximal close to the ICN and one distal close to the AHMM (Figure 3). Histologic examination showed fibrocartilaginous tissue compatible with meniscus in all four biopsies (Figure 3). A complex tear of the body and posterior horn of the medial meniscus was corroborated arthroscopically, and a partial meniscectomy was performed in both knees (Figure 2).

OUTCOME AND FOLLOW-UP

The patient recovered favorably in the postoperative period. The use of crutches for 10 d and physiotherapy for 1 mo was indicated. After a period of strengthening, she returned to sports after 3 mo. Follow-up evaluation 1 year after surgery evidenced full remission of symptoms and the patient had resumed her athletic activities.

DISCUSSION

Variability of the AHMM patterns of insertion is relatively common according to cadaveric and arthroscopic studies[2,3,10]. However, abnormal insertion of the anterior horn into the ICN through the AMMFL is rare. The precise prevalence of this anatomical variation remains underestimated, as it may go undetected on preoperative MRI examination and is often only incidentally discovered during arthroscopy. One of the main issues in our knowledge of the AMMFL is a lack of high-level evidence studies. Our understanding on this topic is largely based on case reports[5,6,9,11-15] and there are only two case series available[7,8]. On the one hand, Kim and Joo[7] performed a retrospective review of 13 patients with an AMMFL with insertion at the ICN. In contrast to our report, all cases were diagnosed incidentally during arthroscopy. Although the AHMM had no bony attachment, they performed an MRI analysis and showed that there was no significant meniscal extrusion. On the other hand, Anderson *et al*[8] published a case series of 12 patients. Six patients were identified on a retrospective review of pictures taken during arthroscopies and the other six patients were identified prospectively. As in our case, all 12 patients had other abnormal conditions that were thought to be the cause of their symptoms.



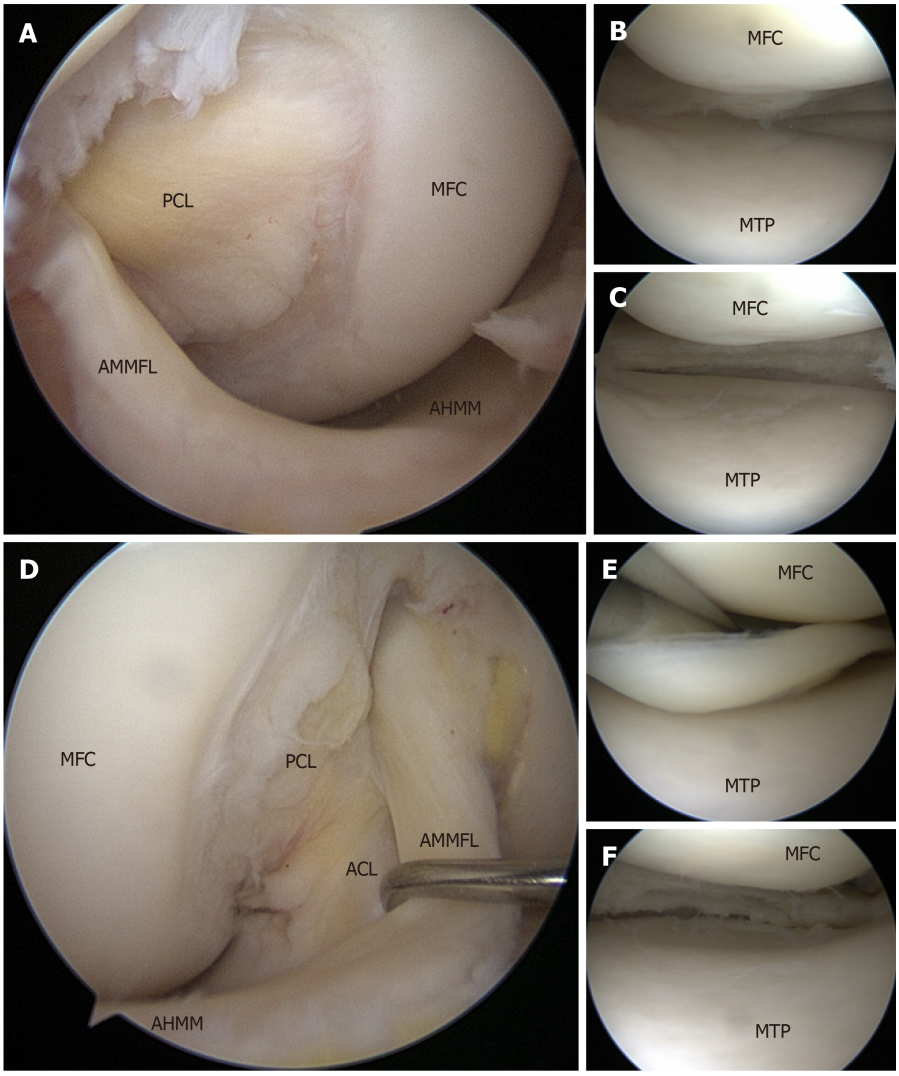
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Figure 1 Magnetic resonance imaging images of the knee. A-D: Magnetic resonance imaging (MRI) images of the right knee. Coronal T2-weighted fat-saturated image demonstrating the anteromedial meniscofemoral ligament (AMMFL) (green arrow) and the distal aspect of the anterior cruciate ligament (ACL) (white asterisk) (A); sagittal T1-weighted image showing the AMMFL (green arrow) running anteriorly to the ACL (white asterisk) (B); coronal T2-weighted fat-saturated and sagittal images showing the medial meniscus with a previous partial meniscectomy and tear of the posterior horn (C and D); E-H: MRI images of the left knee. Coronal T2-weighted fat-saturated image demonstrating the AMMFL (green arrow) and the distal aspect of the ACL (white asterisk) (E); sagittal T1-weighted image showing the AMMFL (green arrow) running anteriorly to the ACL (white asterisk) (F); coronal T2-weighted fat-saturated and sagittal images showing a tear of the posterior horn of the medial meniscus (G and H).

Without prior awareness, the AMMFL may not be identified on preoperative MRI. Our review of the MRI findings revealed comparable features to those previously reported[1,5,16]. The AMMFL was identified as a linear structure of low signal intensity anterior to the ACL on sagittal and coronal views of T1- and T2-weighted images. Among the differential diagnoses, an ACL tear or an infrapatellar plica may be misdiagnosed on sagittal projections and a displaced bucket-handle tear on coronal views[16]. The anatomy of the distal insertion of the AMMFL, along with its morphology and position, can be used to differentiate it from these other structures.

It is still unclear whether the AMMFL may have biomechanical significance. It has been suggested that it functions as an anchor of the AHMM and probably plays an important role in load transmission[8]. This is in good agreement with the results of Kim and Joo[7], in which no significant extrusion of the medial meniscus was observed. However, the lack of normal bony attachment to the tibia causes the meniscus to be hypermobile, resulting in meniscal tears. According to Ohkoshi *et al*[2], during knee flexion, the AMMFL tightens and pulls the AHMM backwards, causing abnormal hoop tension on the entire medial meniscus and potentially inducing progressive degeneration or tears. This is consistent with our finding that the patient had a bilateral AMMFL associated with a torn medial meniscus. There was no impingement against the ICN, therefore, we did not resect the AMMFL and tried to preserve the structure.

Finally, there has been some disagreement regarding histopathological examination of this structure in different studies. Anderson *et al*[8] reported a ligamentous nature in one case, with dense fibrous tissue and collagen interspersed with parallel rows of fibroblasts. In contrast, Nakajima *et al*[9] described the histological features of a meniscal structure.



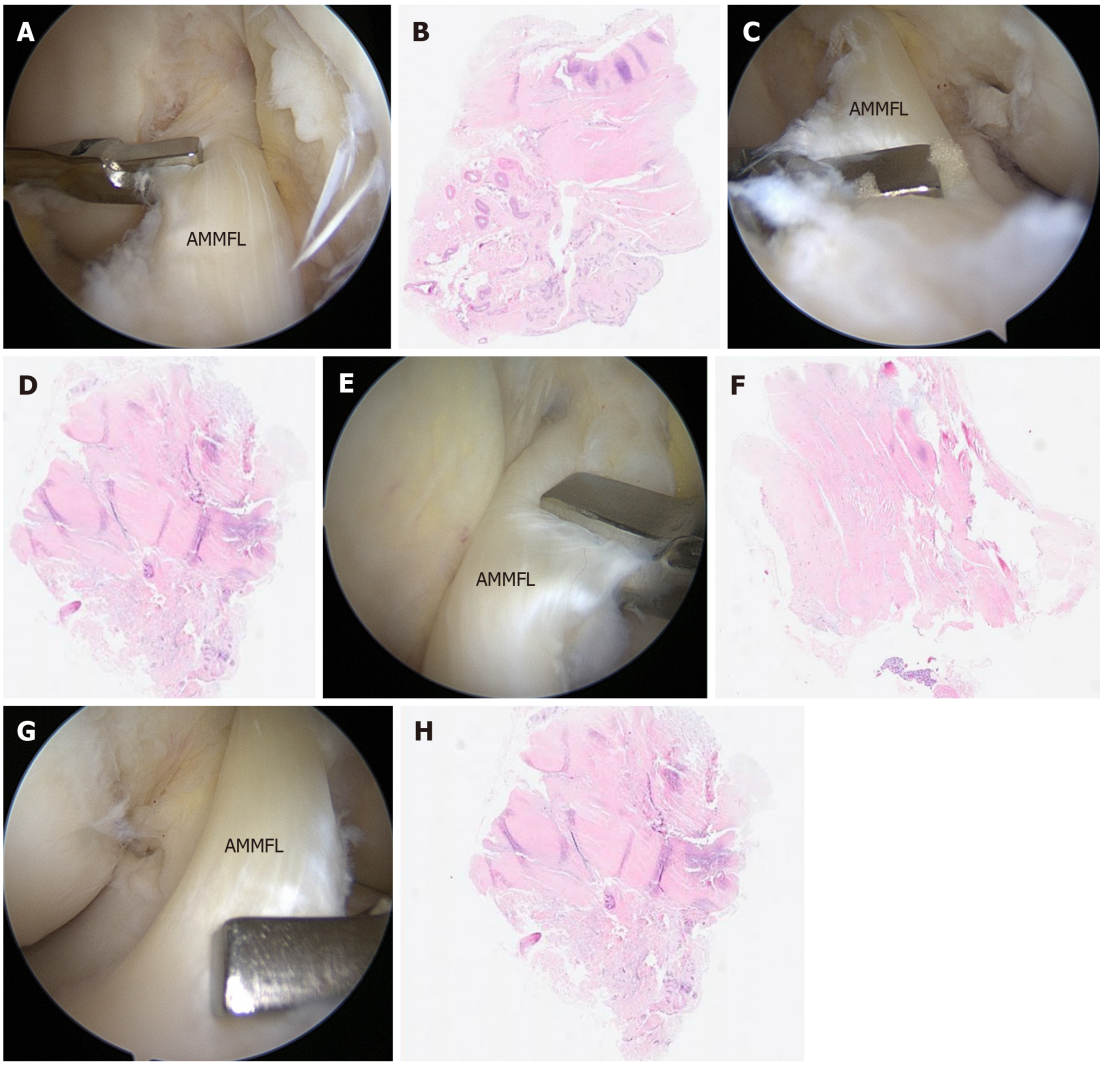
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Figure 2 Arthroscopic images of the knee obtained through the anterolateral portal. A-C: Arthroscopic images of the right knee obtained through the anterolateral portal. The anteromedial meniscofemoral ligament (AMMFL) can be seen coursing anteriorly to the anterior aspect of the anterior cruciate ligament (ACL) and connecting the anterior horn medial meniscus (AHMM) to the posterolateral intercondylar notch (A); tear of the medial meniscus (B); image of the medial meniscus after partial meniscectomy (C); D-F: Arthroscopic images of the left knee obtained through the anterolateral portal. The AMMFL can be seen coursing anteriorly to the anterior aspect of the ACL and connecting the AHMM to the posterolateral intercondylar notch (D); tear of the medial meniscus (E); image of the medial meniscus after partial meniscectomy (F). PCL: Posterior cruciate ligament; MFC: Medial femoral condyle; AMMFL: Anteromedial meniscofemoral ligament; AHMM: Anterior horn medial meniscus; MTP: Medial tibial plateau; ACL: Anterior cruciate ligament.

Some authors argued that this difference could be related to the different biopsy sites. Therefore, we decided to take two biopsies in each AMMFL, one proximal and one distal to study the presence of possible transition zones and be able to define its histological nature. According to our findings, fibrocartilaginous tissue compatible with meniscus was found in all four biopsies.

CONCLUSION

The AMMFL is a very rare entity, commonly unrecognized and underreported. According to our case, as well as other previously published cases, the AMMFL shows several common features that can help reach an accurate diagnosis: (1) The AMMFL is observed as a low signal intensity band-like structure on MRI and is best seen on the sagittal view; (2) It is asymptomatic and often related to meniscal tears; (3) If discovered incidentally during arthroscopy, it should not be removed routinely; and (4) Its histopathological structure is predominantly meniscal tissue.



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Figure 3 Intraoperative images and histologic examination of the knee. A-D: Intraoperative images and histologic examination of the right knee. Arthroscopic images obtained through the anteromedial portal showing the biopsies performed through the anterolateral portal (A and B); hematoxylin and eosin staining of the meniscofemoral band reveals fibrocartilaginous tissue compatible with meniscus in both cases (C and D); E-H: Intraoperative images and histologic examination of the left knee. Arthroscopic images obtained through the anteromedial portal showing the biopsies performed through the anterolateral portal (E and F); hematoxylin and eosin staining of the meniscofemoral band reveals fibrocartilaginous tissue compatible with meniscus in both cases (G and H). AMMFL: Anteromedial meniscofemoral ligament.

FOOTNOTES

Author contributions: Luco JB, Di Memmo D, Nicolino TI, and Garcia-Mansilla I designed the research study; Astou J and Costa-Paz M performed the research; Luco JB and Garcia-Mansilla I analyzed the data and wrote the manuscript; Gomez Sicre V performed the histopathologycal analisis; and all authors have read and approved the final manuscript.

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Country/Territory of origin: Argentina

ORCID number: Matias Costa-Paz 0000-0002-8217-1086; Ignacio Garcia-Mansilla 0000-0002-7247-3734.

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CASE REPORT

Sclerotic marginal zone lymphoma: A case report

Zade Moureiden, Hammad Tashkandi, Mohammad Omar Hussaini

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Zade Moureiden, Hammad Tashkandi, Mohammad Omar Hussaini, Pathology and Laboratory Medicine, Moffitt Cancer Center, Tampa, FL 33612, United States

Corresponding author: Mohammad Omar Hussaini, MD, Associate Professor, Director, Pathology and Laboratory Medicine, Moffitt Cancer Center, 12902 USF Magnolia Drive, Tampa, FL 33612, United States. mohammad.hussaini@moffitt.org

Abstract

BACKGROUND

Marginal zone lymphoma (MZL) is an indolent non-Hodgkin B cell lymphoma with various architectural pattern including perifollicular, follicular colonization, nodular, micronodular, and diffuse patterns. A sclerotic variant has not been previously reported and represents a diagnostic pitfall.

CASE SUMMARY

A 66-year-old male developed left upper extremity swelling. Chest computed tomography (CT) in September 2020 showed 14 cm mass in left axilla. Needle core biopsy of axillary lymph node showed sclerotic tissue with atypical B lymphoid infiltrate but was non-diagnostic. Excisional biopsy was performed for diagnosis and showed extensive fibrosis and minor component of infiltrating B cells. Flow cytometry showed a small population of CD5-, CD10-, kappa restricted B cells. Monoclonal immunoglobulin heavy chain and light chain gene rearrangement were identified. Upon being diagnosed with MZL, patient was treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone and achieved complete remission by positron emission tomography/CT.

CONCLUSION

This is an important case report because by morphology this case could have easily been overlooked as non-specific fibrosis with chronic inflammation representing a significant diagnostic pitfall. Moreover, this constitutes a new architectural pattern. While sclerotic lymphomas have rarely been described (often misdiagnosed as retroperitoneal fibrosis), we do not know of any cases describing this architectural presentation of MZL.

Key Words: Sclerotic; Marginal zone lymphoma; Architecture; Pitfall; Diagnosis; Fibrosis; Case report

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Core Tip: In the clinical context of suspicious lymphadenopathy, the presence of an extensive sclerosis on biopsy should not deter the clinician from a diagnosis of lymphoma, and careful evaluation and work up is needed to exclude covert lymphoma.

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INTRODUCTION

Marginal zone lymphoma (MZL) is an indolent B-cell non-Hodgkin lymphoma derived from marginal zone B cells within the lymphatic system[1]. The incidence of MZL, based on data from the United States SEER-18 program, is 19.6 per 1000000 person-years[2]. MZL is typically classified into extranodal MZL (EMZL) of mucosa-associated lymphoid tissue (61%), nodal MZL (NMZL) (30%), and splenic MZL (SMZL) (9%). Other subtypes include pediatric MZL and immunoproliferative small intestinal disease[3]. 5-year relative survival rate for EMZL, NMZL, and SMZL are 88.7%, 76.5% and 79.7%, respectively with EMZL being most likely to transform to diffuse large B cell lymphoma[4,5].

Marginal zone B cells typically display a morphology of small to medium sized lymphocytes with somewhat irregular nuclei containing mature chromatin and relatively abundant pale cytoplasm. They may classically assume a monocytoid morphology[6]. In SMZL, villous lymphocytes can be seen in the periphery. MZL typically expresses an immunohistochemistry profile positive for B cell-associated antigens (CD19, CD20, CD22, CD79a) and complement receptors (CD21 and CD35). MZLs are usually negative for CD5, CD10, CD23, BCL6, and cyclin D1. Furthermore, SMZL has a high concentration of immunoglobulin D (IgD) cell surface antigens; whereas, EMZL and NMZL show expression of IgM and IgD [3]. MZL can assume various architectural patterns including perifollicular, follicular colonization, nodular, micronodular, and even diffuse patterns[7]. In the bone marrow, an intrasinusoidal pattern is often seen in SMZL[8].

We present a remarkable case of MZL masquerading in a sclerotic background as fibrosis with chronic inflammation. This constitutes the first report of this architectural pattern in MZL and represents a serious and important diagnostic pitfall in lymphoma diagnosis.

CASE PRESENTATION

Chief complaint

The patient is a 66-year-old male who developed left upper extremity swelling.

History of present illness

The patient had an episode of syncope of unclear etiology in January 2020. He started to have pain under his left arm in February that waxed and waned. Then over the course of a few weeks, he started having left upper extremity swelling. The patient had an ultrasound and mammogram that were reportedly negative. This was followed by a computed tomography (CT) of the chest without contrast on April 9, 2020 that showed a large, irregularly marginated mass arising in the left axilla.

History of past illness

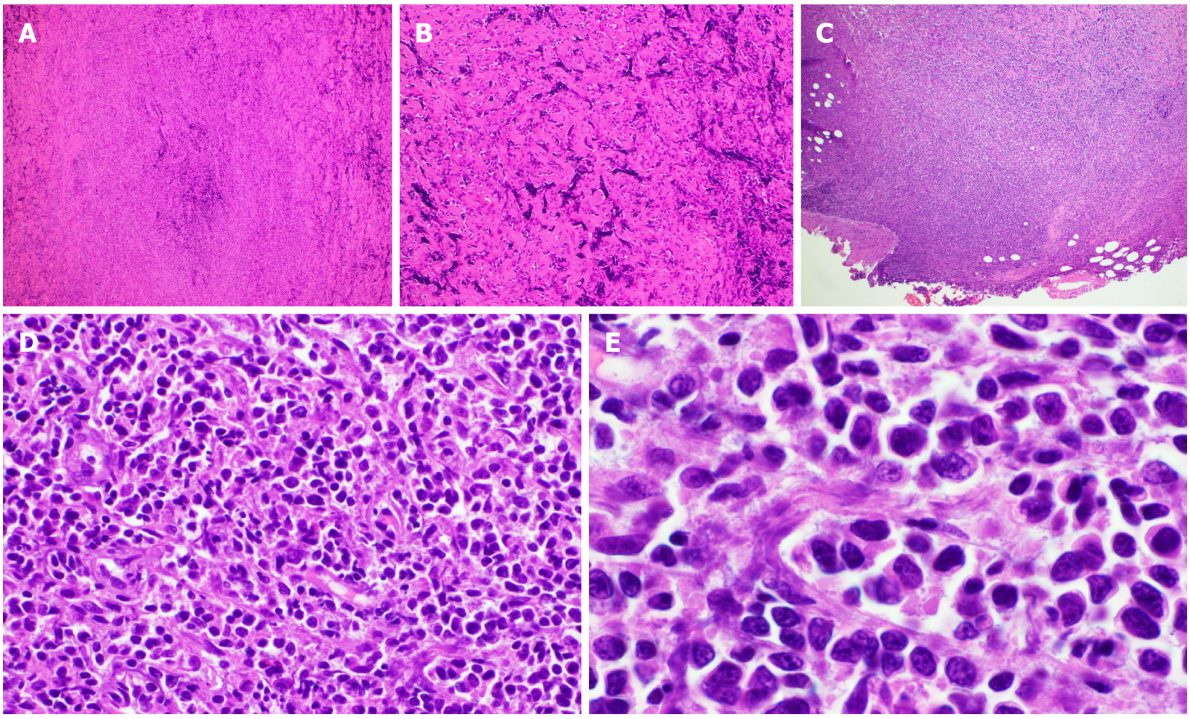
Past medical history is notable for benign prostatic hyperplasia, chronic kidney disease stage III, type 2 diabetes, hypertension, hyperlipidemia, and pulmonary hypertension.

Personal and family history

The patient had a mother with history of breast cancer and colon cancer.

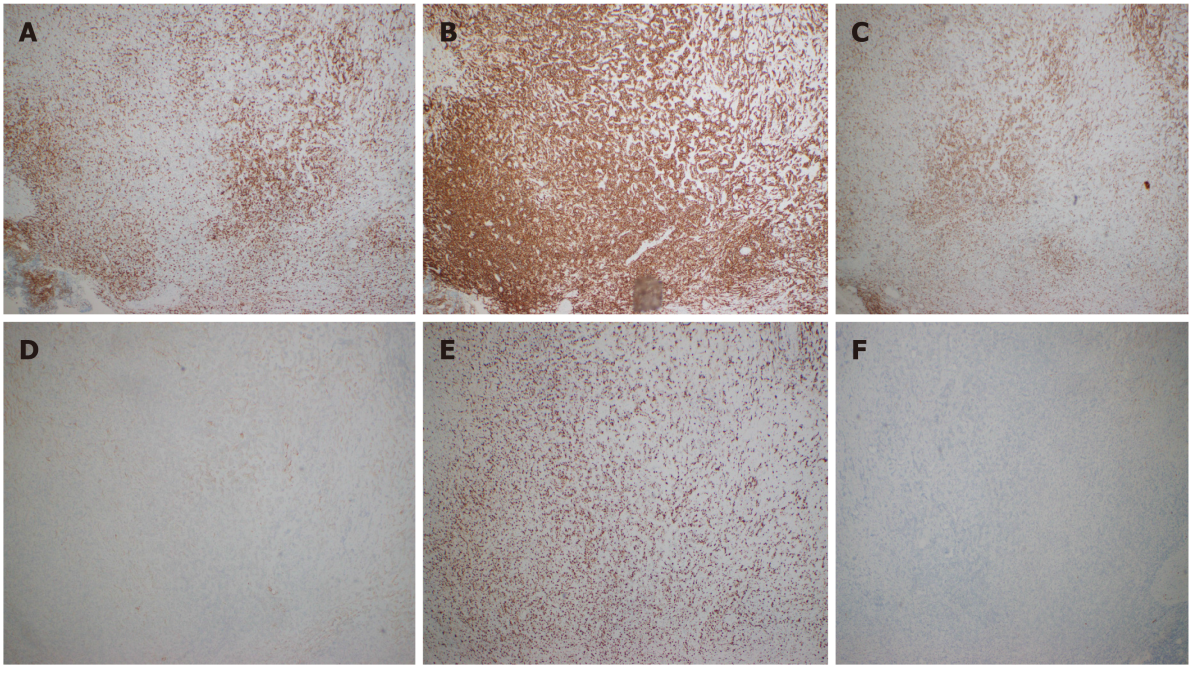
Physical examination

Temperature: 36.78 °C, heart rate: 93/min, respiratory rate: 16/min, blood pressure: 125/73 mmHg, SpO_2: 100%, weight: 104.5 kg, body mass index: 31.20 kg/m^2. General: Alert and oriented, no acute distress. Eye: Normal conjunctivae, anicteric. Head/ears/nose/mouth/throat: Normocephalic, no trauma, normal hearing. Neck: Supple, non-tender. Cardiovascular: Regular rate and rhythm, normal peripheral perfusion. Lung: Lungs were clear to auscultation, respirations were non-labored. Abdomen: Soft, nontender, nondistended, no splenomegaly. Hematological/lymphatics: No lymphadenopathy: Cervical, supraclavicular, axillary, inguinal. Extremities: Normal range of motion, normal strength, no deformity. Integumentary: Warm, dry, intact. Neurologic: Alert, oriented, no focal defects. Cognition and speech: Oriented, speech clear and coherent, functional cognition intact. Psychiatric: Cooperative, appropriate mood and affect.



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Figure 1 Histology of sclerotic marginal zone lymphoma. A: Low power hematoxylin and eosin (H&E) section showing extensive fibrosis and crush artifact, 40 × (Olympus BX43); B: Low power H&E section showing extensive fibrosis and crush artifact, 40 × (Olympus BX43); C: More cellular area with small lymphocytes is seen focally (lower-left hand) compared with more sclerotic pattern (upper-right), 40 ×; D: High power H&E showing morphology of cells in cellular area (non-crushed), some have monocytoid features (400 ×); E: High power H&E showing morphology of cells in cellular area (non-crushed), some have monocytoid features (1000 ×).



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Figure 2 Immunohistochemical profile of sclerotic marginal zone lymphoma. A: CD3; B: CD20; C and D: CD5; E: Ki67; F: BCL6.

Laboratory examinations

The specific examinations and results are listed in Table 1. Pathology results are provided in Figures 1-3.

Table 1 Laboratory examinations

	Value w/units	Normal range
CBC		
WBC	5.59 k/uL	4.00-10.90
Preliminary ANC	> 1.5 k/uL	
RBC	3.50 mil/uL	4.45-5.73
Hemoglobin	11.1 g/dL	13.4-16.9
Hematocrit	34.1%	40.0-48.0
Mean cell volume	97.4 FL	80.3-94.0
MCH	31.7 pg	27.4-33.4
MCHC	32.6 g/dL	32.0-36.8
RDW	50.6 FL	36.8-46.1
Platelet count	113 k/uL	143-382
MPV	10.1 FL	7.4-11.7
Differential		
Neutro auto	3.57 k/uL	1.80-7.80
Eos auto	0.37 k/uL	0.00-0.45
Basophil auto	0.04 k/uL	0.00-0.20
Immature Gran auto	0.03 k/uL	0.00-0.10
Mono auto	0.71 k/uL	0.30-0.80
Lymph auto	0.87 k/uL	1.10-3.50
Nucleated RBC	0.00 k/uL	0.00-0.10
Differential %		
Neutro auto %	63.9	
Eos auto %	6.6	
Basophil auto %	0.7	
Immature Gran auto %	0.5	
Mono auto %	12.7	
Lymph auto %	15.6	
Nucleated RBC %/100 WBC	0.0/100 WBC	0.0-1.0
Coagulation		
Prothrombin time	11.6 s	10.2-12.9
INR	1.0	0.8-1.1
APTT	27.0 s	25.1-36.5
Reticulocyte percent	1.78%	0.80-1.90
Reticulocyte number	0.0623 mil/uL	0.0360-0.1000
Immature retic fraction (%)	14.5	3.0-13.4
Eryth. sed rate	20 mm/hr	0-15
Metabolic panel		
Sodium	140 mmol/L	134-145
Potassium	4.0 mmol/L	3.4-4.5
Chloride	102 mmol/L	96-107
Total CO_2	26 mmol/L	22-30

Glucose level	111 mg/dL	70-110
BUN	13 mg/dL	6-23
Creatinine	1.5 mg/dL	0.7-1.3
Est. GFR	47 mL/min/1.73 m^2	
Est. GFR (Af-Am)	57 mL/min/1.73 m^2	
Uric acid	6.9 mg/dL	3.5-8.5
Calcium	11.3 mg/dL	8.6-10.2
Calcium corrected	11.2 mg/dL	8.6-10.2
Phosphorus	2.7 mg/dL	2.5-4.5
Total protein	6.8 gm/dL	6.6-8.7
Albumin	4.1 gm/dL	3.5-5.2
Total bilirubin	0.80 mg/dL	0.00-1.20
Alk. phosphatase	65 U/L	40-130
AST	34 U/L	10-50
ALT	25 U/L	0-41
LDH	158 U/L	135-225
Magnesium level	1.4 mg/dL	1.6-2.3

CBC: Complete blood count; WBC: White blood cell; ANC: Absolute neutrophil count; RBC: Red blood cell; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red blood cell distribution width; MPV: Mean platelet volume; INR: International normalized ratio; APTT: Activated partial thromboplastin time; BUN: Blood urea nitrogen; GFR: Glomerular filtration rate; AST: Aspartate aminotransferase; ALT: Alanine transaminase; LDH: Lactate dehydrogenase.

Imaging examinations

Chest CT in September 2020 showed a 14 cm irregularly, marginated mass arising in left axilla. There was questionable invasion of the left subscapularis muscle and thickening the right pectoralis minor muscle. Positron emission tomography (PET) scan showed standardized uptake value (SUV) of 26.

FINAL DIAGNOSIS

The final diagnosis is MZL.

TREATMENT

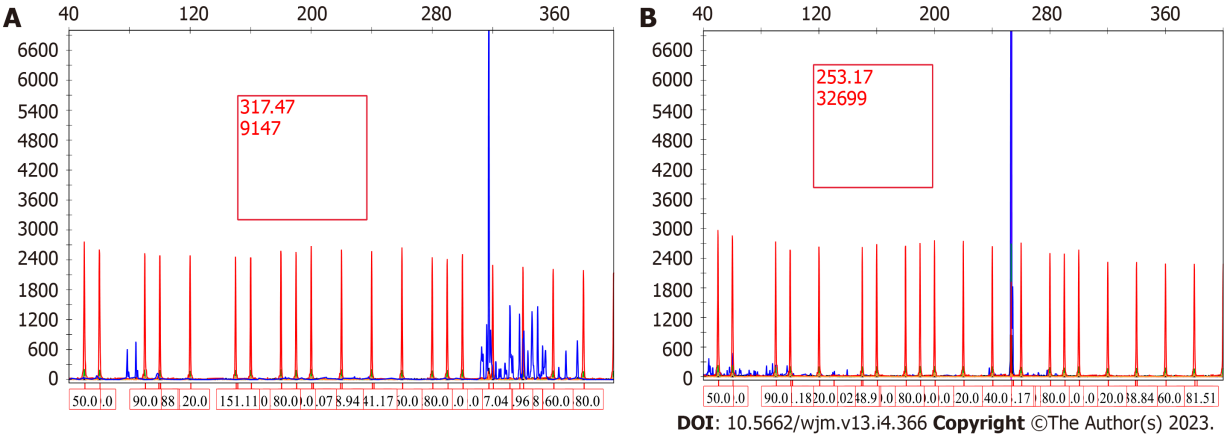
Upon being diagnosed with lymphoma, patient was treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). More specifically, he received 6 cycles of R-CHOP, and doxo/cyclophosphamide 50% dose reduction starting cycle 4 due to severe cytopenias.

OUTCOME AND FOLLOW-UP

The patient achieved complete remission by PET/CT (July 20, 2021). He is under surveillance post treatment and 1-year post-treatment scans show no evidence of disease.

DISCUSSION

MZL can demonstrate a wide spectrum of clinical manifestations due organ-specific variability. Genomically, there is also heterogeneity, although dysregulation of B-cell receptor, nuclear factor κB, and NOTCH signaling pathways is typical[3]. Significant variation is also seen in the architectural patterns manifested by MZL[7]. This is complicated by the fact that there is no single universal biomarker for MZL, and the diagnosis is often only arrived at after integrating phenotypic,



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Figure 3 Clonal peaks detected in immunoglobulin heavy chain. A: Framework 1 region; B: Framework 2 region.

cytogenetic, and molecular features[9].

There are a few studies that have cataloged the architectural patterns in MZL. Salama *et al*[10] evaluated 51 NMZL and found four major patterns, namely: Diffuse (75%), well-formed nodular/follicular (10%), interfollicular (14%) and perifollicular (2%). Interestingly, they noted compartmentalizing interstitial sclerosis in 28% of cases most commonly in the diffuse variant (12/15 cases). However, this was illustrated as relatively inconspicuous comprising of delicate tendrils of sclerosis which in no way resembles our case. Others have documented variable architectural patterns depending on the site of involvement: Spleen (nodular to diffuse), bone marrow (intrasinusoidal, interstitial, nodular, and even paratrabecular), lymph node (nodular to diffuse, liver (intrasinusoidal and portal tract lymphoid nodules), *etc*[9].

Rarely, lymphomas can present in a markedly fibrotic background or be clinically misdiagnosed as retroperitoneal fibrosis[11,12]. Sclerosing lymphomas are rare and typically of follicle center origin[13]. We do not know of any cases describing this architectural presentation of MZL. As such, this case could have easily been overlooked as non-specific fibrosis with chronic inflammation representing a significant diagnostic pitfall. Clues with regard to the diagnosis include the radiologic findings of a large (14 cm) mass with high SUV. Furthermore, the B cell predominance by immunohistochemistry was atypical. Flow cytometry and immunoglobulin heavy and light chain gene rearrangement studies were vital in order to arrive at the correct diagnosis. Response to R-CHOP further confirms the diagnosis clinically.

CONCLUSION

In summary, we present the first case report of sclerotic MZL which should be recognized as a rare architectural pattern in MZL and poses a diagnostic challenge, especially on limited fine-needle aspiration or needle core biopsy specimens. Integration of all clinical and pathological data is essential to arrive at the correct diagnosis.

FOOTNOTES

Author contributions: Moureiden Z, Tashkandi H, and Hussaini MO contributed to the conception and design of the study, acquisition and analysis of data; Moureiden Z and Hussaini MO drafted the manuscript or figures.

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ORCID number: Mohammad Omar Hussaini 0000-0001-5192-0489.

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Editorial Board Member of *World Journal of Methodology*, Rodrigo Valenzuela, PhD, Associated Professor, Department of Nutrition, Faculty of Medicine, University of Chile, Independence Av. 1027, Santiago 8380000, Chile. rvalenzuelab@med.uchile.cl

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Ibraheem M Alkhawaldeh, Ibrahem Albalkhi, Abdulqadir Jeprel Naswhan

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Ibraheem M Alkhawaldeh, Faculty of Medicine, Mutah University, Karak 61710, Jordan

Ibrahem Albalkhi, Department of Neuroradiology, Alfaisal University, Great Ormond Street Hospital NHS Foundation Trust, London WC1N 3JH, United Kingdom

Abdulqadir Jeprel Naswhan, Nursing for Education and Practice Development, Hamad Medical Corporation, Doha 3050, Qatar

Corresponding author: Abdulqadir Jeprel Naswhan, MSc, RN, Director, Research Scientist, Senior Lecturer, Senior Researcher, Nursing for Education and Practice Development, Hamad Medical Corporation, Rayyan Road, Doha 3050, Qatar. anashwan@hamad.qa

Abstract

Oversampling is the most utilized approach to deal with class-imbalanced datasets, as seen by the plethora of oversampling methods developed in the last two decades. We argue in the following editorial the issues with oversampling that stem from the possibility of overfitting and the generation of synthetic cases that might not accurately represent the minority class. These limitations should be considered when using oversampling techniques. We also propose several alternate strategies for dealing with imbalanced data, as well as a future work perspective.

Key Words: Machine learning; Class imbalance; Overfitting; Misdiagnosis

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Core Tip: Addressing class imbalance in medical datasets, particularly in the context of machine learning applications, requires a cautious approach. While oversampling methods like synthetic minority oversampling technique are commonly used, it is crucial to recognize their limitations. They may introduce synthetic instances that do not accurately represent the minority class, potentially leading to overfitting and unreliable results in real-world medical scenarios. Instead, we can consider exploring alternative approaches such as Ensemble Learning-Based Methods like XGBoost and Easy Ensemble which have shown promise in mitigating bias and providing more robust performance. Collaborating with data science specialists and medical professionals to design and validate these techniques is essential to ensure their reliability and effectiveness in medical applications.

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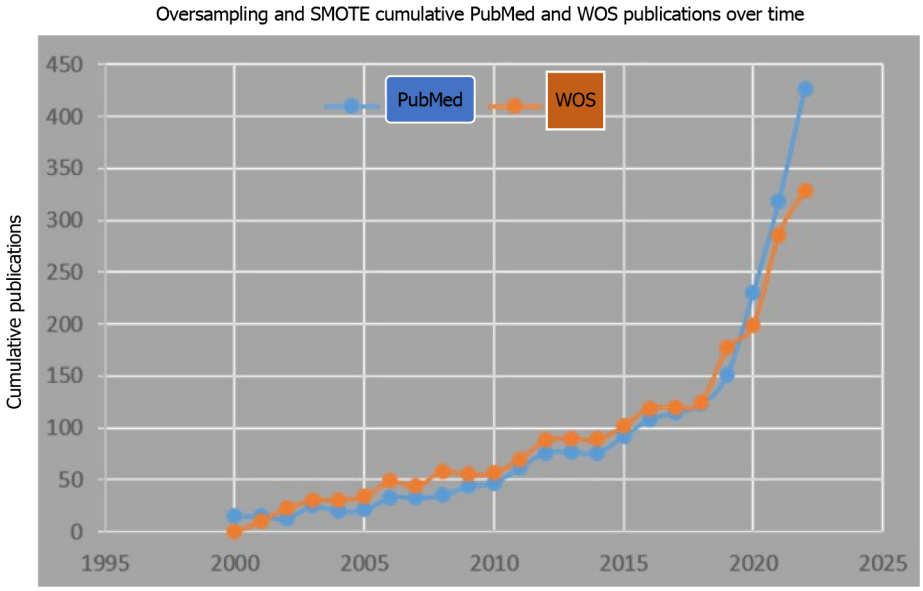
INTRODUCTION

Imbalanced medical data may have a variety of issues that impede classification, such as the impact of noisy data and borderline samples, class overlapping, class imbalance, or the presence of small disjuncts. When training a dataset, an imbalanced class distribution can occur when one class has significantly more samples than the other, resulting in a majority and minority class. This class imbalance can lead to prediction bias in machine learning models, which often translates to poor performance in the minority class(es). To address this issue, several techniques have been proposed in the literature. These techniques include increasing the number of samples from the minority class by obtaining more data from the source, modifying the loss function to assign a higher cost to misclassifications in the minority class, oversampling the minority class by replicating or generating synthetic samples, undersampling the majority class by reducing the number of instances, or using a combination of these approaches. By employing these techniques, the aim is to mitigate the class imbalance problem and improve the performance of machine learning models on imbalanced datasets[1]. There are benefits and drawbacks to each strategy. Many publications have used oversampling, particularly synthetic minority oversampling technique (SMOTE) approaches to create artificial samples from minority samples, to address the issue of class imbalance in the medical, biomedical, and life sciences fields. This is evident from the abundance of oversampling methods developed in the last two decades. For instance, a PubMed search for the terms "oversampling" OR "SMOTE" produced 2157 results from publications between 2000 and 2022, whereas a Web of Science search produced 2185 hits (but only for medical-related topics) (Figure 1). Additionally, when comparing this to undersampling using a PubMed search for ("undersampling" and "machine learning") and ("oversampling" or "SMOTE" and "machine learning"), a noticeable difference is found (Figure 2). This essentially indicates the developing trend of oversampling research in the medical literature, which dealt with or simply discussed oversampling.

Although there has been a substantial increase, it is important to note that this does not automatically imply the effectiveness of the oversampling approach. The surge in oversampling research can be attributed to the significant prevalence of the class imbalance problem and the relative simplicity of oversampling solutions[2]. The concern regarding oversampling methods arises from their potential to artificially increase the number of minority-class instances by generating new ones based solely on their similarity to existing minority examples. This raises concerns about the possibility of overfitting during the learning process. While oversampling techniques may yield favorable results in machine learning experiments, this does not necessarily translate to practical success. Additionally, a more significant issue with oversampling is that the synthetic examples created may actually belong to a different class in the real world, despite their similarity to the minority class examples. This is due to the fact that there are instances from class A that are closer to examples from a different class B, regardless of their similarity to the minority class examples[3].

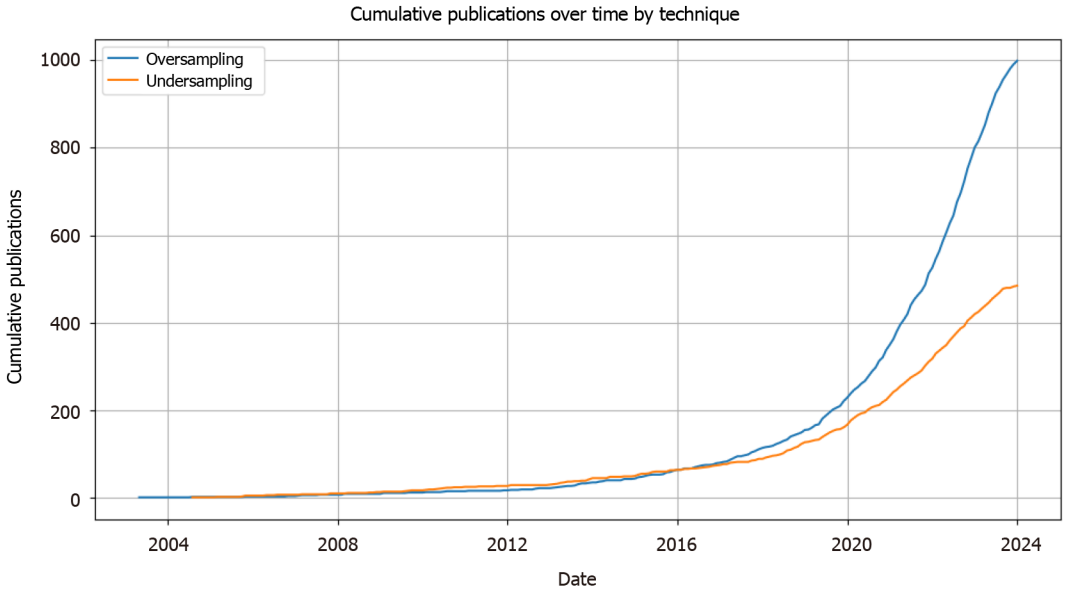
Multiple experimental papers have provided evidence to support the concerns regarding oversampling methods. Elreedy *et al*[4] conducted a study where they analyzed the probability distribution of the synthetic samples generated by the SMOTE method. Their findings led them to conclude that the synthetic data produced by SMOTE may not precisely match the original distribution of the minority class, which can have an impact on the classification performance. Similarly, Tarawneh *et al*[2] argue against the current forms and methodologies of oversampling, considering it a deceptive approach. They suggest that oversampling introduces falsified instances that are falsely classified as members of the minority class when they are more likely to belong to the majority class. Their conclusions were drawn from a recommended validation system that was applied to various class-imbalanced datasets, including medical datasets. The validation system involved hiding a number of majority examples and assessing the similarity between the synthesized examples generated by different oversampling methods and the hidden majority examples[3].

After conducting a detailed analysis of their findings, it becomes evident that all validated oversampling approaches suffer from errors in the synthesized samples. These approaches generate instances that are intended to represent the minority class but actually resemble the majority class or fall within the decision boundary of the majority class (Figure 3). The error rate varies across different validated methods and oversampled datasets, ranging from 0% to 100%. None of the strategies achieve zero error on all datasets, indicating their inability to accurately oversample medical records. The oversampling techniques attempt to fill the feature space gap by creating new instances that are similar to



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Figure 1 The number of papers that discussed, utilized, or addressed oversampling or synthetic minority oversampling technique between 2000 and 2022.

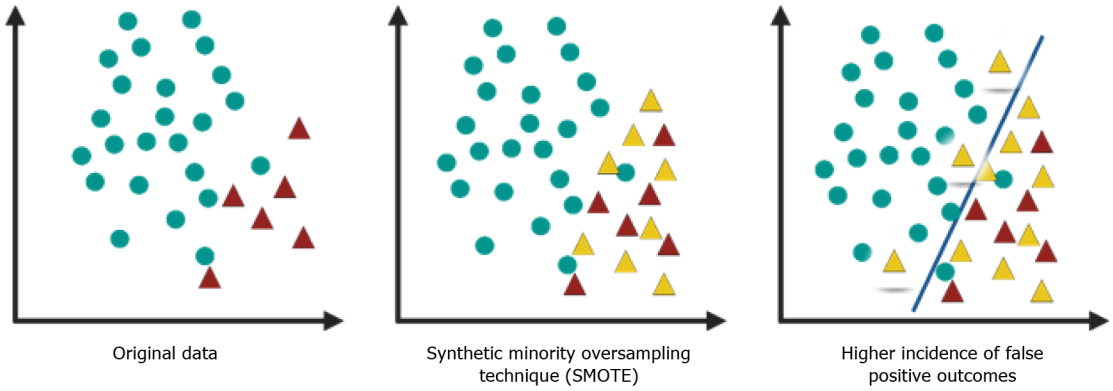


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Figure 2 Trend of PubMed publications on oversampling and undersampling techniques in machine learning over the years.

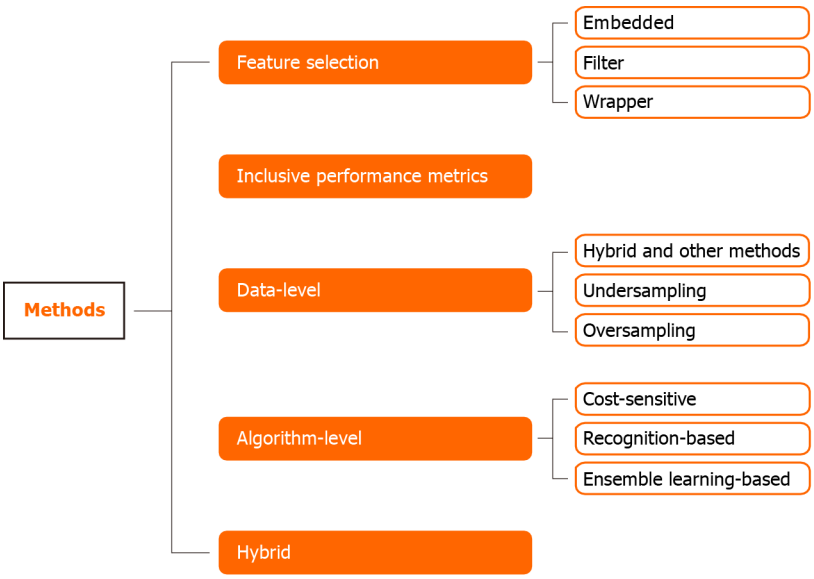
one or more minority instances. However, these techniques wrongly assume that the synthesized examples belong to the minority class without providing any guarantee. Consequently, the training of these instances becomes misguided, increasing the risk of overfitting the classifier on false data. This poses a significant threat as the entire machine learning system may fail when applied in real-world medical applications, where even a single incorrectly generated example can have severe consequences. Therefore, oversampling structured medical datasets by synthesizing new instances solely based on their resemblance to the minority examples is a questionable practice, particularly in the context of medical data. It is crucial to ensure that the additional samples truly fall within the minority class. Moreover, since the model itself is flawed, any external validation, subsequent analysis, or conclusions based on it should be critically examined. The potential harm and consequences of a misdiagnosis, inaccurate prediction, or prognosis can be particularly detrimental for cancer patients[1].

When it comes to the currently existing methods for dealing with class imbalance problems, researchers suggested various strategies for analyzing data, which are classified as data level, algorithm level, and hybrid (Figure 4). The methods are dependent on the size of the data collection, distribution, imbalance ratio, and model performance criteria[5].



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Figure 3 Errors in the synthetic minority sample after synthetic minority oversampling technique application. SMOTE: Synthetic minority oversampling technique.



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Figure 4 Overview of the existing methods for the class imbalance problem.

Undersampling approaches, like oversampling, have some drawbacks at the data level, such as the loss of critical information for data distribution. Undersampling can result in the loss of relevant information by removing valuable and significant patterns. Oversampling and undersampling approaches can be used independently or in hybrid methods. Because they are based on existing techniques, these hybrid methods built on them share the same limitations[5]. Recent research suggested random partitioning of data with a voting rule, a resampling method that works by randomly splitting the imbalanced dataset into a number of smaller balanced sub-datasets. On each sub-dataset, a machine-learning model is subsequently trained. The final prediction is made by applying a voting mechanism to the individual model forecasts. Other resampling strategies were outperformed by this strategy. When tested using several machine learning classifiers on 33 benchmark class-imbalanced datasets, this approach has the potential to overcome the present limitations [6].

Hybrid techniques combine methods at multiple levels. For example, when data-level methods are used to process data externally and distribute classes to instances, the learning process is then carried out internally using algorithm-level methods. You can read for a thorough explanation of these techniques[7]. In algorithm-level methods, researchers have the ability to modify conventional machine learning models by assigning weights or costs to classifiers in order to mitigate bias towards the majority class. This approach ensures that the learning model remains unaffected by the class distribution. These methods can be categorized as recognition-based, cost-sensitive, or ensemble learning-based techniques.

In the absence of non-target class instances, recognition-based approaches such as one-class learning are employed. They model the classifier on the representation of the minority class and proceed to learn primarily from minority class instances rather than attempting to distinguish dissimilar patterns from majority class and minority class examples. One-class classification includes features such as outlier identification and novelty discovery. This approach performs well, particularly with high-dimensional data. One-class learning may be used to build many models, including support vector

machines and isolation forests; however, it cannot be used to build other models, such as decision trees and Nave Bayes [5].

Cost-sensitive methods are crucial in medical applications because of the significance of minimizing false positive and false negative instances. These methods involve adjusting the misclassification cost to achieve a balance between the majority and minority classes. For instance, assigning a higher weight or cost to false negative predictions compared to false positive predictions can be an effective approach. This practical solution enables cost-sensitive learning in the context of medical applications. In the literature, there are several cost-based approaches available to address class imbalance in data, including weighted cross-entropy, multiclass dice loss function, and focal loss. There are other recent methods such as weighted extreme learning machine, cost-sensitive decision tree ensemble methods, and cost-sensitive deep neural networks[5,8].

Ensemble learning methods have gained significant attention in various fields, including machine learning and medical applications. These methods combine multiple weak learners to create a more robust model with improved performance. Some popular ensemble learning techniques including voting and boosting ensemble learning models, such as XGBoost and Easy Ensemble, have been found to outperform individual learning models and exhibit greater resistance to noise and outliers[3,8]. However, it is important to consider the potential drawbacks of ensemble learning. These models often require significant training time and may be prone to overfitting in certain scenarios. To address the limitations of ensemble learning, researchers have introduced new techniques. Ensemble pruning methods aim to reduce the complexity and training time of ensemble models while maintaining their performance. Regularization techniques, such as dropout and bagging, have also been incorporated into ensemble models to mitigate the risk of overfitting[8].

The use of feature selection approaches is growing in addressing data imbalance. These techniques reduce computational and storage costs, eliminate redundant information, and facilitate data visualization. Feature selection methods can be grouped into filter, wrapper, and embedded methods. Filter methods select variables using statistical measures, while wrapper methods assess features based on model performance and selection criteria. Embedded methods, like least absolute shrinkage and selection operator regression, perform feature selection as an integral part of the learning process [5]. Additionally, it is important to consider diverse performance metrics, such as the area under the precision-recall curve, Matthew's correlation coefficient, F-score, and geometric mean, to effectively evaluate model performance in the presence of class imbalance. These metrics provide a comprehensive assessment of model performance, taking into account precision, recall, and the balance between sensitivity and specificity[5,9,10].

To effectively address class imbalance, it is advisable to consider various approaches at different levels, including data-level, algorithm-level, and hybrid-level techniques. These approaches aim to mitigate bias and improve classifier algorithms by combining different methods. There is a growing body of research focusing on hybridization techniques that integrate sampling, feature selection, and classifier building to gain a better understanding of class representation and achieve more accurate classification results. For instance, evolutionary computing can be employed in feature selection, while ensembles can be constructed to tackle the challenges associated with class imbalance. By adopting these practical and reliable solutions, the issue of class imbalance in medical datasets can be significantly improved, as these approaches are based on sound assumptions[5,9].

To gain insights into the impact of oversampling and other methods on real-world medical applications, it is crucial to collaborate with data science specialists and medical professionals. By working together, these experts can create and evaluate these approaches, taking into account their unique perspectives and expertise. This collaboration can provide valuable guidance and influence the selection or adaptation of appropriate techniques. The joint efforts of medical doctors and data scientists can lead to the development of more reliable and efficient solutions in the field of healthcare.

CONCLUSION

It is recommended to address class imbalance at different levels considering data-level, algorithm-level, and hybrid-level approaches that can mitigate bias. At the algorithmic level, Ensemble Learning-Based Methods such as XGBoost and Easy Ensemble, have proved to have a better performance than individual learning models, and they provide more resistance to noise/outliers. Another hybrid method is Random Undersampling Boost which is not without limitations either, noting that there is not a one-size-fits-all approach and exercising caution should be taken when addressing class imbalances. The adoption of such more practical and trustworthy solutions would improve the class imbalance issue in medical datasets more because these approaches have no wrong assumptions[2-6]. It is vital to understand how oversampling and other methods may affect real-world medical applications. Collaborating with data science specialists and medical professionals can enhance the development and testing of reliable and effective solutions, as their insights can provide valuable advice and influence the selection or modification of relevant techniques.

FOOTNOTES

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Country/Territory of origin: Qatar

ORCID number: Ibraheem M Alkhawaldeh 0000-0002-0187-1583; Abdulqadir Jeprel Naswhan 0000-0003-4845-4119.

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EDITORIAL

Current protocol to achieve dental movement acceleration and pain control with Photo-biomodulation

Angela Dominguez

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Peer-review model: Single blind

Peer-review report's scientific quality classification

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Grade B (Very good): 0
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Angela Dominguez, Department of Orthodontics, Faculty of Dentistry, Benemerita Universidad Autónoma de Puebla, Zaragoza de Puebla Calle 4 Sur 104, Puebla, C.P. 72000, Mexico

Corresponding author: Angela Dominguez, DDS, Researcher, Department of Orthodontics, Faculty of Dentistry, Benemerita Universidad Autónoma de Puebla, Zaragoza de Puebla Calle 4 Sur 104, Puebla, C.P. 72000, Mexico. angela.dominguezc@gmail.com

Abstract

When designing a study on dental movement acceleration or pain control during orthodontic treatment, it is crucial to consider effective parameters. The objective of this editorial is to compile the most effective parameters supported by evidence that should be considered in future studies to achieve complete parameter homogenization. The protocol currently recommended to homogenize the parameters and facilitate the development of further meta-analysis in terms of acceleration of movement and pain control in orthodontics is Wavelength: 810 nm, 2.2 J per surface, 0.1 W in continuous mode/0.1 W average power in a super-pulsed, sweeping movement, 1mm from the mucosa, 22 seconds along the vestibular surface and 22 seconds along the lingual surface, the recommended speed of movement is 2 mm/sec, 1 application during each orthodontic control, to achieve dental movement acceleration and repeat the dose at 24 h to ensure pain elimination. The energy density and power density will depend on the spot size used in the equipment and the distance from the mucosa. It will strengthen the evidence of photobiomodulation as the best therapy to accelerate tooth movement and at the same time control the pain produced by orthodontic treatments.

Key Words: Photobiomodulation; Laser-assisted orthodontics; Dental movement acceleration; Pain control; Diode laser

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Core Tip: Several Systematic Reviews and Meta-Analyses have been published to evaluate the effectiveness of photobiomodulation (PBM) in accelerating dental movement and pain control during orthodontic treatment. These studies suggest that PBM is an effective method to achieve these objectives. However, all reports show a lack of standardization in the ideal parameters required.

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INTRODUCTION

It is very common to find in randomized controlled clinical trials that evaluate the speed of tooth movement during orthodontic treatments or pain control, diverse protocols of photobiomodulation (PBM).

This means that at the time of developing a meta-analysis, or even a systematic review, the authors report that there needs to be a consensus in respect of guidance for clinical use, due to the lack of consistency of wavelengths and parameters applied.

The research question is no longer whether PBM controls pain and accelerates tooth movement when applied during orthodontic treatment. At the moment several systematic reviews and meta-analyses are in support of its efficacy[1-7].

The current question is how can we obtain the greatest effectiveness in PBM when we want to achieve those objectives.

To accomplish this goal, studies should focus on determining the necessary parameters such as wavelength, energy, power, application time, energy density, application points, and number of sessions.

This editorial aims to synthesize the parameters that are currently accepted to be effective in achieving these objectives during orthodontic treatment:

Pain control and dental movement acceleration.

Employing standardized parameters for accelerating movement and pain control will allow clinicians to be more effective in achieving these goals and will allow researchers to have adequate reproducibility in randomized controlled clinical trials that will later form part of systematic reviews and meta-analyses.

Wavelength

It is not enough to reproduce parameters such as energy, power, time, and energy density if we select a wavelength that is not the most effective to accelerate the movement.

Do we know what it is? The answer is: Yes.

In 2020, the first systematic review was conducted to establish an effective range of low-level laser therapy wavelengths for accelerating tooth movement in orthodontic treatment[8]. That review concluded that most randomized controlled trials related to accelerating tooth movement in orthodontic treatments are ideally between 780 and 830 nm wavelengths. The mean increase in speed of the dental movement calculated as a percentage of the control group was 24%.

Three years later, a systematic review and meta-analysis were performed with the same objective[9]. The authors concluded that ≤ 810 was determined as the wavelength associated with faster orthodontic tooth movement.

A range that fluctuates between 730 nm to 830 nm stimulates cell activity better[10,11]. It has been reported that wavelengths over 900 nm attract melanin and hemoglobin, resulting in a superficial energy uptake in soft tissue and insufficient energy in bone tissue. Shorter wavelengths have a greater ability to pass through soft tissue. Additionally, wavelengths within the range of 618 to 405 nm may not effectively stimulate orthodontic movement[12].

As for the effective wavelength to control pain during orthodontic treatments, the evidence has shown that pain reduction after 24 h is not significantly dependent on wavelength[13].

This indicates that if we are applying PBM to control pain and it disappears, we are not necessarily using the most effective wavelength to accelerate movement, as we considered in the years before these publications.

Emission mode

Until a few years ago, most studies were carried out to obtain the benefits of PBM in continuous mode. Most of the literature, before 2015, where we referred to Low-Level laser Therapy, provided studies with therapeutic equipment that emitted in continuous or pulsed mode. Currently, we have super-pulsed lasers available. However, the emission mode in laser-assisted orthodontics is not decisive. After selecting a device with the recommended wavelength, it can be continuous, pulsed, or super-pulsed. It is essential to take this into account when calculating the total energy delivered.

Power

Several studies report 100 mW (0.1 W)[14-18], however, this varies greatly depending on the equipment used. Nowadays there is equipment that operates in pulsed or continuous mode, but its minimum power is 0.2 W, so it can be pulsed at 50% to obtain an adequate amount of total energy. We also have super-pulsed diodes in which it is advisable to select 0.2 W.

Total energy

Since 2014 thanks to the first systematic review that evaluated these two objectives[19], we know that the most common and effective energy input is in the range of 0.2-2.2 J per point/2-8 J per tooth, to accelerate orthodontic tooth movement and control pain, the recommended energy per point ranged from 1-2 J when a single tooth was irradiated to 0.5-2.25 J per point when all teeth in the dental arch were irradiated.

Currently, 2-4 J per tooth is suggested to obtain both effects.

Energy density

Energy density is the most important parameter in laser PBM, as it estimates the actual energy received by the target tissue. It is often referred to as fluence (J/cm^2), meaning dose; however, some scholars also refer to energy in joules as dose[20].

The energy density is frequently mentioned in the dental literature, but the area of the spot in the tissue is often omitted. This error makes it impossible to verify their results or to see how they calculated the critical energy density information. Inconsistency in reporting these parameters is a major source of conflicting results in research and has contributed greatly to hindering acceptance of the effects of PBM[21].

The energy density is equal to the total energy if the tip or handpiece used in the equipment measures 1 cm 2, however, the dental equipment used for intraoral applications has round tips and in many cases, very small used without activation to allow the energy to reach the tissue and obtaining much higher energy densities, which are not always reported and make it difficult to standardize the parameters at the time of meta-analysis. This is why the ranges are extensive and energy density intervals range from 4.25 to 80 J/cm^2; depending on the tip used to achieve movement acceleration and pain control.

Equipment tips are not interchangeable between manufacturers.

To adapt the protocol in the clinic, depending on the equipment that the practitioner has, he can adjust the energy density in 80 J/cm^2 by applying the formula:

$$DE = \frac{P(W) \times T(s)}{A\ (cm^2)}$$

DE: Energy density;

T: Time in seconds;

A: Area in cm^2 in cm^2;

It is important to take into account that manufacturers report the diameter of the tip. To find the area in cm^2, the following formula should be applied:

$A = \pi \times r^2$

Examples: For a 7 mm diameter tip the area is 0.38 cm^2; For a 3 mm diameter tip the area is 0.07 cm^2.

Ideally, spot size (The area that reaches the tissue) measurements would be preferred, but due to the complexity of clinical practice due to is an infrared laser, it is recommended to standardize applications based on tip area, which is the closest alternative.

Power density

Aside from energy density, a second important parameter in laser measurement is power density[22]. Few studies have reported on power density, thus it is not extensively covered in the literature: 20 mW/cm^2[23] and 6.37 W/cm^2[24].

The energy density is not adequately reported because the distance from the mucosa to which the laser is applied is not standardized and this parameter depends on this distance.

Currently, it is suggested at 1mm from the mucosa.

Future studies should report without exception the energy density to facilitate a later consensus on both energy density and power density (fluence and irradiance) when looking for either of the two therapeutic objectives during orthodontic treatment.

Punctual application or scanning movement

The average increase in speed movement calculated as a percentage of the control group found in 2020 was 24%. Many authors recommended punctual application and divided the root into thirds, for example, Limpanichkul *et al*[14] and Doshi-Mehta *et al*[16], and some up to 5 points per surface[25,26]. This type of application is highly recommended in research cases to calculate the energy density per point in a precise way. However, it is complicated to reproduce it in clinical practice and it is easier to make a scanning movement. The recommended speed of movement is 2 mm/sec[27]. During orthodontic treatment, this movement is performed along the buccal and lingual surface of each tooth which requires acceleration for 22 s to obtain 2.2 J per surface, at 100 mW (0.1 W)[28] obtaining an average 30% acceleration of the movement. These parameters are also used for pain control.

Number of sessions

Studies differ greatly in the number of applications. There are reports of several acceleration appointments between orthodontic controls[29-31]. It is advisable to apply PBM in the orthodontic control appointment without additional sessions; repeated doses between control appointments do not increase the average acceleration.

The increase in the number of sessions does not exceed 30% acceleration of the movement which is the percentage by which the speed is increased if applied just in the controls. Therefore, it is not justifiable to increase the number of appointments between them.

To guarantee a pain-free orthodontic treatment, it is necessary to repeat the dose 24 hours after the orthodontic adjustment[32]. The suggested single monthly dose that allows acceleration of movement is insufficient to eliminate pain.

CONCLUSION

The protocol currently recommended to homogenize the parameters and facilitate the development of further meta-analysis in terms of acceleration of movement and pain control in orthodontics is Wavelength: 810 nm, 2.2 J per surface, 0.1 W in continuous mode/0.1 W average power in a super-pulsed, sweeping movement, 1mm from the mucosa, 22 seconds along the vestibular surface and 22 seconds along the lingual surface, the recommended speed of movement is 2 mm/sec, 1 application during each orthodontic control, to achieve dental movement acceleration and repeat the dose at 24 h to ensure pain elimination. The energy density and power density will depend on the spot size used in the equipment and the distance from the mucosa.

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FOOTNOTES

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Country/Territory of origin: Colombia

ORCID number: Angela Dominguez 0000-0002-6434-1969.

S-Editor: Liu JH
L-Editor: A
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EDITORIAL

New evidence-based practice: Artificial intelligence as a barrier breaker

Ricardo Maia Ferreira

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Ricardo Maia Ferreira, Department of Sports and Exercise, Polytechnic Institute of Maia (N2i), Maia 4475-690, Porto, Portugal

Ricardo Maia Ferreira, Department of Physioterapy, Polytechnic Institute of Coimbra, Coimbra Health School, Coimbra 3046-854, Coimbra, Portugal

Ricardo Maia Ferreira, Department of Physioterapy, Polytechnic Institute of Castelo Branco, Dr. Lopes Dias Health School, Castelo Branco 6000-767, Castelo Branco, Portugal

Ricardo Maia Ferreira, Sport Physical Activity and Health Research & Innovation Center, Polytechnic Institute of Viana do Castelo, Melgaço, 4960-320, Viana do Castelo, Portugal

Corresponding author: Ricardo Maia Ferreira, PhD, Professor, Sports and Exercise Department (N2i), Polytechnic Institute of Maia, Avenida Carlos de Oliveira Campos, Maia 4475-690, Porto, Portugal. rferreira@ipmaia.pt

Abstract

The concept of evidence-based practice has persisted over several years and remains a cornerstone in clinical practice, representing the gold standard for optimal patient care. However, despite widespread recognition of its significance, practical application faces various challenges and barriers, including a lack of skills in interpreting studies, limited resources, time constraints, linguistic competencies, and more. Recently, we have witnessed the emergence of a ground-breaking technological revolution known as artificial intelligence. Although artificial intelligence has become increasingly integrated into our daily lives, some reluctance persists among certain segments of the public. This article explores the potential of artificial intelligence as a solution to some of the main barriers encountered in the application of evidence-based practice. It highlights how artificial intelligence can assist in staying updated with the latest evidence, enhancing clinical decision-making, addressing patient misinformation, and mitigating time constraints in clinical practice. The integration of artificial intelligence into evidence-based practice has the potential to revolutionize healthcare, leading to more precise diagnoses, personalized treatment plans, and improved doctor-patient interactions. This proposed synergy between evidence-based practice and artificial intelligence may necessitate adjustments to its core concept, heralding a new era in healthcare.

Key Words: Evidence; Clinicians; Patients; Artificial intelligence; Evidence-based practice

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Core Tip: Evidence-based practice principles remain crucial in clinical care. However, practical application faces challenges. The recent emergence of artificial intelligence offers solutions for the main barriers. Artificial intelligence can swiftly provide evidence, enhances clinical decision-making, combat patient misinformation, and improve clinical consultations. The integration of artificial intelligence into evidence-based practice represents a potential paradigm shift, requiring some adjustments to the core concept of evidence-based practice.

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INTRODUCTION

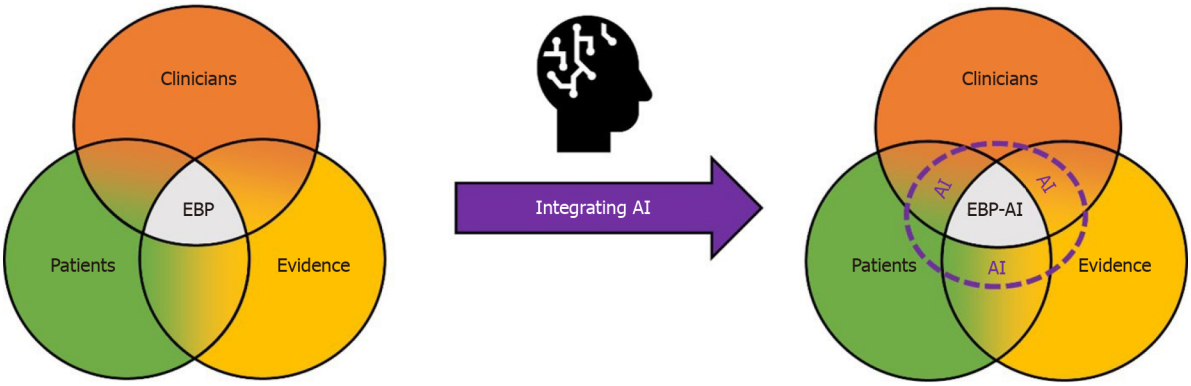
The evidence-based practice (EBP) principles are several years old[1], yet their importance remains as relevant as ever. EBP continues to be considered the gold standard for the best clinical practice[2]. As it was developed some years ago, it has undergone some changes (*e.g.*, evidence-informed practice), but its foundation principles persist, with the best evidence, clinical expertise, and patient preferences playing central roles[3]. However, despite the widespread acknowledgment of its importance in the daily clinical practice, its practical application can be challenging and some barriers to its implementation arise. Some of these barriers include[4]: Lack of skills to understand studies; insufficient resources and funding; time constraints; lack of informatics and linguistic competencies; overtasking, heavy workload and competing priorities; inadequate training and stakeholders support; patient personal characteristics and health illiteracy; lack of motivation, confidence, interest, and commitment to change; evidence "unrealistic", inaccessible, "unreadable", conflicting, and massive.

While the concept is not new[5-9], we have only recently witnessed the emergence, interest, and adoption of a new technological revolution known as artificial intelligence (AI)[5,10]. AI encompasses the creation of machine learning technology, capable of performing high-level executive functions that typically require human intelligence (such as reasoning, learning, planning, and creativity)[7,11-17]. We observe applications in techs (*e.g.*, smart cars, smart homes, smartphones, computers, robots, and drones), navigation systems, code writing, facial recognition, chatbots, image and data analysis, translators, audio output, and more[11-13,18-23]. Like any other technological tool, AI has incrementally become a part of our lives, despite the reluctance of some public[13,16,24,25]. The AI use brings both advantages and disadvantages. Its advantages and disadvantages are summarized in (Table 1)[7,14,24,26-35].

By understanding and carefully evaluating its pros and cons, AI could be considered as a potential solution for overcoming some of the main barriers encountered in the application and implementation of EBP. Here are some examples: (1) Inability to stay updated with the best evidence. As it may be known, the pace of scientific production is currently at its peak. The number of articles is growing exponentially[36], making it nearly humanly impossible to search for and read all the information published every day. AI can assist in the search process by summarizing the latest literature within seconds, thus saving clinicians time for other tasks[24,26,31,37-39]. However, as it currently stands, AI still has some "bugs" (known as *AI Hallucinations*) and is not yet able to critically analyze it[12,13,21,34,37,39-51], making it essential that the clinician continues to do their own "homework"[51,52]. In addition, there is another evidence-related barrier – comprehension. Studies often employ a specialized scientific language, with English as the predominant language[53,54]. Many clinicians still do not have a satisfactory scientific and linguistic understanding to stay updated [55]. AI is already capable of providing definitions, complete document translations, (re)writing, and summarizing[13,18,21,22,25,37,39,54,56-61], overcoming this barrier; (2) Enhanced clinical decision-making. During a clinical session, it is up to the clinician to assess the patient's clinical situation and, based on the results, present the best intervention plan to their patients, considering the best and most recent literature along with their clinical experience[62]. Therefore, the first phase of clinical reasoning heavily relies on the clinician's "isolated" judgment. AI has already evolved to the point where it can integrate information from imaging and clinical findings, typical disease progression patterns, treatment responses (risk and benefits), and scientific information[7,8,12,16,23,24,35,38,39,57,63-70]. Consequently, AI can act as a second clinician in the decision-making process, where the human clinical expert can interact with the "artificial clinical expert", leading to more accurate decisions and presenting more precise diagnoses, prognoses, and personalized/tailored interventions plans for their patients[6,14,16,23,52,57,65,69-73]. This human-machine interaction may be particularly valuable for those just starting out their careers. As explored, one of the foundations for better clinical decision-making involves clinical experience. However, those who are just starting out in the profession do not yet have enough clinical experience to be experts in the field, and often have to make the first stage of the decision solely based on scientific evidence[74]. Therefore, AI could act as the expert in this situation, helping novice clinicians with their clinical decision-making; (3) Patient misinformation. While patients often do not actively participate in clinical decision-making, their beliefs and preferences should, in accordance with the principles of EBP, be considered when devising an intervention plan[1]. In this way, there is a mutual partnership between the clinician and the patient, as their beliefs and preferences can help

Table 1 Example of artificial intelligence advantages and disadvantages

Advantages	Disadvantages
Efficiency	Job displacement
Accuracy	Bias
Cost reduction	Lack of empathy
Constant availability	Complexity
Data analysis	Security risks
Customization	Lack of transparency
Scalability	Fairness
Natural language processing	Regulation
Automation	Ethical concerns
Productivity	
Accessibility	



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Figure 1 Evidence-based practice artificial intelligence. EBP: Evidence-based practice; AI: Artificial intelligence.

(positively) limit the number of possible interventions/therapies/treatments, making a treatment plan easier to adhere [75]. However, patients' beliefs and preferences can sometimes pose a barrier. Patients frequently arrive at clinical appointments misinformed about their clinical condition and some of the interventions/therapies/treatments[76]. AI, if less biased[34,40], could correctly provide information to patients about their clinical condition and the most appropriate interventions/therapies/treatments[14,71,77]. This will greatly facilitate the doctor-patient interaction, thus enhancing the quality of intervention planning and clinical management[71,77]; and (4) Time constraints in clinical practice and consultations. As is well known, many clinicians spend a substantial amount of time on bureaucratic and administrative tasks, leaving them with insufficient time for proper patient care[7,38,75]. AI can assist in scheduling, triage, filling out forms, billing, monitoring, and responding to routine tasks (almost like an artificial assistant or secretary)[7,28,38,69,73,78]. This would allow clinicians to free up more time for tasks that involve essential human interaction, simply by issuing a few basic and quick commands[31,38,52].

CONCLUSION

As explored, AI can be considered a useful tool in clinical management, encompassing various aspects such as time management, assessment, interaction, prescription, monitoring, decision-making, information processing, and more. This could potentially usher in a paradigm-shift in EBP, requiring minor adjustments to its core concept. Consequently, the new EBP-AI proposal is presented in (Figure 1).

FOOTNOTES

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Country/Territory of origin: Portugal

ORCID number: Ricardo Maia Ferreira 0000-0003-1596-9685.

Corresponding Author's Membership in Professional Societies: Polytechnic Institute of Maia; Polytechnic Institute of Coimbra; Polytechnic Institute of Castelo Branco; Sport Physical Activity and Health Research & Innovation Center.

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OPINION REVIEW

Evidence-based literature review: De-duplication a cornerstone for quality

Barbara Hammer, Elettra Virgili, Federico Bilotta

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Barbara Hammer, Librarian at Medical Library, University of Bergen, Bergen 5020, Norway

Elettra Virgili, Federico Bilotta, Anesthesiology, Critical Care and Pain Medicine, University of Rome "La Sapienza", Rome 00166, Italy

Corresponding author: Federico Bilotta, MD, PhD, Professor, Anesthesiology, Critical Care and Pain Medicine, University of Rome "La Sapienza", viale del Policlinico, 155, Rome 00166, Italy. federico.bilotta@uniroma1.it

Abstract

Evidence-based literature reviews play a vital role in contemporary research, facilitating the synthesis of knowledge from multiple sources to inform decision-making and scientific advancements. Within this framework, de-duplication emerges as a part of the process for ensuring the integrity and reliability of evidence extraction. This opinion review delves into the evolution of de-duplication, highlights its importance in evidence synthesis, explores various de-duplication methods, discusses evolving technologies, and proposes best practices. By addressing ethical considerations this paper emphasizes the significance of de-duplication as a cornerstone for quality in evidence-based literature reviews.

Key Words: Duplicate publications as topic; Databases; Bibliographic; Artificial intelligence; Systematic reviews as topic; Review literature as topic; De-duplication; Duplicate references; Reference management software

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Core Tip: Effective de-duplication is crucial for maintaining the quality and credibility of systematic reviews. It ensures data accuracy, eliminates bias, reduces workload, and enhances trust in findings. However, challenges such as variability in data, database indexing, and resource constraints exist. Best practices include clear documentation, the use of reference management software, manual review when necessary, handling multiple versions of the same paper, addressing non-journal sources, and ethical considerations. Advancements like Deduklick and Automated Systematic Search Deduplicator offer promise for more accurate and efficient de-duplication methods. De-duplication is a fundamental step in evidence synthesis, contributing to transparent and reproducible research in systematic reviews.

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INTRODUCTION

Evidence-based literature reviews are essential for informed decision-making in research and practice. However, without proper de-duplication, duplicated records may skew findings, leading to biased conclusions. This opinion review aims to shed light on the importance of de-duplication in evidence synthesis and its evolution over time and to give a big picture of what's available as a solution so research teams can make an informative decision on what's best for the project when it comes to de-duplication.

What is de-duplication?

In the world of computing, database de-duplication refers to the technique of ensuring that specific information is only stated once. One way to find information that is consistent across multiple sources (such as data files, books, websites, and databases) is through record linkage (RL). Data matching, RL, data linkage, entity resolution, and many other terms are focused on finding such records and eliminating duplicates. "RL is necessary when joining different data sets based on entities that may or may not share a common identifier (*e.g.*, database key, URI, National identification number), which may be due to differences in record shape, storage location, or curator style or preference"[1]. The term de-duplication, in medical scientific writing, refers to the process of identifying and removing duplicate citations from the search results retrieved from various databases. "Record de-duplication is of great advantage for de-duplicating citation in bibliographic databases"[2]. However, the identification of duplicated citations is not a trivial task. "Records are usually not identical, because they may come from different databases and may differ in the treatment of authors' names of journal titles, indexing, and special field"[3]. Duplicated citations are the result of the standard in evidence synthesis studies like systematic reviews which require comprehensive searching in multiple databases to identify eligible studies. Duplicates can arise due to multiple factors, such as the same study being indexed in multiple databases and because "certain types of information are recorded differently (and inconsistently) in the different databases"[4].

As stated by the research team from the Pain Research, Nuffield Department of Anaesthetics, Churchill Hospital in Oxford: "if duplicate records are not removed effectively, reviewers can waste time screening the same records for inclusion and run the risk of accidentally including same paper more than once in their meta-analyses, leading to inaccurate conclusions"[5]. Hence removing duplicate citations is an important and necessary step between searching and screening in a process of the systematic review.

In practice, de-duplication is available *via* search platforms, reference management software (Table 1), and screening assistance tools (Table 2). However, automation does not entirely solve de-duplication issues. The process typically involves exporting search results into the library in one of the research management tools, merging data sets and identifying duplicated citations. Duplicates are identified by comparing various bibliographic elements such as titles, authors, publication dates, journal names, *etc.* Once potential duplicates are identified, the research team reviews these records to confirm if they are indeed duplicates. Confirmed duplicates are removed from the data set, ensuring that only unique study is kept and counted only once in the systematic review. Then the de-duplicated data set forms the basis for subsequent stages of the systematic review: study selection, data extraction, and data synthesis.

EVALUATION OF THE DE-DUPLICATION - METHODS, TECHNIQUES, AND TOOLS

De-duplication can be traced back to various stages in the development of information management and technology. Over time, de-duplication methods have evolved from manual to sophisticated automated techniques. However, to this day effective de-duplication methods may involve a combination of automated and manual approaches to ensure accurate and reliable results, as the race to create the ultimate tool continues. "In the early days of bibliographic record-keeping manual cataloging was the only process of creating metadata representing information resources, such as books, sound recordings, moving images, *etc.* Cataloging provided information such as author's names, titles, and subject terms that describe resources, typically through the creation of bibliographic records"[6]. Librarians and researchers manually

Table 1 Reference management software

Paid	Free
EndNote	Mendeley
RefWorks	Zotero
	Bib TeX

Table 2 Systematic review tools that offer de-duplication

Paid	Free
Covidence	Rayyan
DistillerSR	Systematic Review Assistant-De-duplication Module
Deduklick	Automated Systematic Search Deduplicator

reviewed catalogue cards or printed bibliographies to identify and eliminate duplicate entries. "The introduction of the term database coincided with the availability of direct-access storage from the mid-1960s onwards. As computers grew in speed and capability, several general-purpose database systems emerged; by the mid-1960s a number of such systems had come into commercial use"[7]. "Since the 1970s metadata were in machine-readable form and were indexed by information retrieval tools, such as bibliographic databases or search engines"[6].

With the rise of electronic databases, de-duplication has become more complex. Databases allowed for the storage and retrieval of vast amounts of bibliographic information, leading to an increased need for automated methods in de-duplication. There are several existing de-duplication tools, methods, and techniques that have been developed to address the challenges of identifying and eliminating duplicates from datasets. "Reference management software has been a useful tool for researchers since the 1980s"[8]. Within a brief timeframe, a marketplace was established, and commercial products were produced. The development of *e.g.*, EndNote[9], Zotero[10], or Mendeley[11], *etc.* provided researchers with tools to manage and de-duplicate their collections of references. This type of tool introduced automated (default) de-duplication features.

The introduction of Digital Object Identifiers (DOIs) "in the late 1990s, and implementation in the early 2000s"[12], has greatly facilitated de-duplication. DOIs provide a standardized and unique identifier for each publication, making it easier to track and manage duplicates. Screening assistance tools like, *e.g.*, Covidence[13] and Rayyan[14] were developed specifically for systematic reviews. These tools integrated automated de-duplication, collaboration features, and support for the review process. "Modern data matching algorithms utilize advanced techniques, including tokenization, stemming, and phonetic algorithms, to handle variations in text data and improve the accuracy of matching.

The following terms explain the various types of de-duplication processes: (1) Exact match de-duplication: This method examines precise matches in key fields, such as unique identifiers or customer IDs. If the same information is shown on multiple records, these duplicates are removed; (2) Fuzzy match de-duplication: Fuzzy de-duplication techniques use algorithms to determine the similarity between records, even if they do not have exact matches in key fields, allowing for the recognition of duplicates with slight differences or misspellings; and (3) Rule-based de-duplication: Rule-based de-duplication involves defining specific rules or criteria to identify duplicates. These rules can be based on data patterns, business logic, or specific requirements"[15].

Practical solutions for effective de-duplication constantly evolve. In 2013 research team from Fourth Military Medical University in China described a pragmatic strategy of combining automated and manual searching duplicates in a systematic review[16]. This paper evaluates the extensiveness and characteristics of duplicates in the PubMed, EMBASE, and Cochrane Library databases. Identifies two types of duplicates: Type-I (duplicates among different databases) and type-II (duplicate publications in different journals/issues). Results showed that most type-I duplicates are identified by the auto-searching method, while nearly all type-II duplicates are identified by the hand-searching method. The hand-searching approach has a substantially greater incidence of incorrect items in type-I duplicates, most of which come from the EMBASE database. The authors recommend employing a combined strategy of auto-and-hand-searching methods to find duplicates in the systematic review due to the insufficiency of a single strategy.

In 2015, Canadian researchers explored and compared the effectiveness of various de-duplication features, specifically in the Ovid and EBSCO database platforms and three selected reference management software packages: RefWorks, EndNote, and Mendeley[17]. The authors recorded the time taken to de-duplicate each option, the number of false positives, and the false negatives, and in conclusion, recommended different de-duplication options based on the skill of the searcher and the reason for de-duplication. Overall, the results of the study highlight the variation in time and effectiveness of different de-duplication options, providing insights for researchers to choose the most suitable option based on their needs and expertise. Same year research team from Bond University in Australia developed a de-duplication program - The Systematic Review Assistant-De-duplication Module (SRA-DM) to improve the effectiveness of duplicate detection[18]. The paper presents the evaluation of the SRA-DM against EndNote's default de-duplication process, comparing their sensitivity and specificity in detecting duplicates. The goal of the study was to determine the reliability and effectiveness of the SRA-DM in removing duplicate records. In conclusion, SRA-DM demonstrated superior

sensitivity (84%) and specificity (100%) compared to EndNote's default de-duplication process, resulting in a 42.86% increase in the number of duplicate records detected. The paper acknowledged that no software can currently detect all duplicate records, and there are limitations to the SRA-DM, such as undetected duplicates due to discrepancies in data and extraneous information inserted into the title field.

In 2016, an international research team led by Erasmus MC-Erasmus University Medical Centre in Rotterdam developed a de-duplication method (colloquially referred to as the Bramer method) for de-duplicating database search results in EndNote, a popular reference manager, which is used by information professionals conducting exhaustive searches for systematic reviews[19]. The authors highlight the limitations of relying on unique identifiers like DOIs and PMIDs for identifying duplicates and propose using pagination as an alternative. They discuss the variations in page number formats used in different databases and provide a method for adapting the page number format of references to facilitate de-duplication. The paper addresses the challenges of existing de-duplication methods, which are time-consuming or impractical, and compares different software programs. The authors provide detailed instructions for customizing EndNote settings, creating export files with expanded page numbers, and installing filters for importing modified files. Overall, the paper contributes a practical and efficient method for de-duplicating database search results in EndNote, addressing the limitations and challenges of existing methods. This method is still very popular even though it was introduced in 2016.

In 2019, two researchers from the university library at the Vrije University in Amsterdam created AMSTERDAM EFFICIENT DE-DUPLICATION (AED) METHOD. The paper describes the authors' method of de-duplication, which provides a systematic approach to de-duplicating articles and claims to be 100% reliable[20]. The AED method explains per database/host what steps are needed to successfully de-duplicate data sets. This multi-step approach for efficient de-duplication includes collecting accession numbers during the initial search which is useful for an update search and then followed by manual assessment. If the data set is large authors advise following up with the Bramer method.

In 2021, Canadian researchers evaluated the accuracy and efficiency of commonly used electronic methods for flagging and removing duplicate references in systematic reviews[21]. Testing included the default settings (using the default algorithm of each program) in Ovid multifile search, EndNote desktop, Mendeley, Zotero, Covidence, and Rayyan. A benchmark set of unique, de-duplicated references was created through manual abstraction, and the performance of different de-duplication methods was compared against this benchmark set. The study identifies Ovid, Covidence, and Rayyan as the most accurate methods for identifying duplicate references, with Ovid and Covidence having high specificity and Rayyan demonstrating high sensitivity. The paper highlights the strengths and weaknesses of commonly used de-duplication methods and provides strategies for improving their performance to avoid unintentionally removing eligible studies and introducing bias into systematic reviews. The limitation of this paper is the fact that it does not provide specific details about the number of false-negative and false-positive duplicate references for each method or the overall accuracy, sensitivity, and specificity values. Still, the findings of the study are important for researchers in selecting database platforms and supporting software programs for conducting systematic reviews, highlighting those factors such as availability, ease of use, functionality, and capability must be taken into consideration.

In 2022, researchers were introduced to the most advanced de-duplication to date. The Swiss research team from the University of Bren has developed an automated, artificial intelligence-based algorithm named "Deduklick" which combines natural language processing algorithms with a set of rules created by expert information specialists[22]. This automated de-duplication uses a multistep algorithm of data normalization, calculates a similarity score, and identifies unique and duplicate references based on metadata fields, such as title, authors, journal, DOI, year, issue, volume, and page number range. Authors claim that the algorithm significantly reduced the time spent on analysis, simplifying the systematic review process. The performance was comparable to expert information specialists while preserving high metadata quality. The algorithm's transparent and explainable decision process, along with its reproducibility and adherence to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standards[23], makes it a reliable tool for duplicate removal. Although this sounds groundbreaking, this paper is not free from limitations *e.g.*, it does not address potential biases or limitations in the algorithm's decision-making process, such as the impact of different metadata fields on duplicate detection or does not discuss potential limitations or challenges in implementing Deduklick in real-world systematic review processes. Also, the evaluation of the performance was limited to eight existing datasets, and it is unclear if these datasets represent the full range of systematic review scenarios.

In 2023, research team from the University of Edinburgh developed Automated Systematic Search Deduplicator (ASySD) an automated tool to conduct the de-duplication of systematic searches in biomedical databases[24]. In this paper, the authors compared ASySD with other existing tools, such as EndNote's default de-duplication option and the SRA-DM. As a result, ASySD outperformed the alternative methods, correctly removing > 95% of duplicate citations across five biomedical datasets, while removing a few citations incorrectly (specificity > 0.999). The paper's limitations are - the tool was only developed using preclinical systematic review datasets and its performance in other review areas has not been fully evaluated. The accuracy of ASySD depends on the quantity and quality of citation information, and it may not work well with older searches or citations lacking certain bibliographic information. ASySD may mistakenly remove some citations, which is a concern for smaller reviews where each relevant paper is important. Additionally, the memory requirements for larger datasets exceed what is possible on shinyapps.io, so users may need to run the Shiny application locally in R Studio, which can be challenging for non-R-proficient individuals.

IMPORTANCE OF DE-DUPLICATION IN EVIDENCE SYNTHESIS

De-duplication serves as an important step in evidence-based literature reviews hence research teams must be aware of

the two types of de-duplication in medical scientific writing[25]. As mentioned earlier the first type exists on a database level and is a result of the event when a single manuscript was concurrently entered into two or more databases. Usually detected *via* automatic de-duplication. The second type exists between various journals when a single manuscript/study was released in several publications/issues/editions. This category is often referred to as study de-duplication "as it aims to identify two distinct reports of the same study. Study deduplication, although the rarer of the two is only usually detected after data have been extracted from both papers, after the authors have been contacted, or sometimes not at all" [26]. Usually detected *via* screening in full text. Removing duplicate records is essential in maintaining data accuracy and integrity, as they can introduce inaccuracies and redundant data extraction and analysis efforts. De-duplication streamlines the process by focusing on unique evidence, minimizing confusion and misinterpretation in systematic reviews. It eliminates bias and overestimation as duplicates can bias the results of evidence synthesis by inflating the apparent number of studies available for analysis. "Using a primary study results multiple times in the same analysis overstates its sample size and number of events, falsely leading to greater precision in the analysis"[27]. It enhances the quality of synthesis as evidence synthesis aims to provide a comprehensive and accurate overview of the available evidence. "If the same study has more than one report - possibly with different author lists, different titles, and in different journals - both papers should often be cited, but they should only be included in the meta-analysis as one trial" [26,28]. De-duplication ensures that the synthesis is based only on a unique and high-quality set of studies. Reduce workload and improve efficiency as removing duplicates reduces the workload for reviewers and analysts, allowing them to focus on analysing unique studies. This enhances the efficiency of the evidence synthesis process. Provide transparent and reproducible research as de-duplication is an integral part of transparent and reproducible research. Documenting the process ensures that others can replicate the de-duplication and validation steps, enhancing the credibility of the review. Align with publication standards and guidelines as many publication guidelines, including the PRISMA, emphasize the importance of de-duplication to maintain the quality and credibility of systematic reviews. Most importantly enhances trust in findings as de-duplication increases the trustworthiness of the systematic review findings by demonstrating a rigorous and transparent approach to handling data.

WHAT ARE THE CHALLENGES AND LIMITATIONS OF DE-DUPLICATION IN SYSTEMATIC REVIEWS?

De-duplication in systematic reviews, while necessary, is not without challenges and limitations which were touched on earlier. It is important to be aware of these potential issues to effectively address them and ensure the accuracy and reliability of the review process. Conducting manual reviews to confirm duplicates is time-consuming, especially when titles and abstracts do not provide enough information for differentiation. Managing and processing large complex data sets manually can be time-consuming, and error-prone, automated de-duplication seems to be the best option however deciding on either manual or automated de-duplication requires careful consideration.

Variability in data contributes to the issue - variations in study titles, author names, and other bibliographic elements can complicate the de-duplication process as it makes it difficult to accurately identify duplicates requiring careful consideration of matching criteria.

Differences in database indexing - different databases use varied indexing and citation formats which leads to inconsistent or incomplete data *e.g.*, page numbers, which can affect how duplicates are identified, making it harder to determine if two records are indeed duplicates. Furthermore, as a result of those discrepancies automated de-duplication tools may produce "false negatives (duplicate citations that should have been deleted but were not) and false positives (duplicate citations that were deleted but should not have been)"[17]. Hence researchers need to account for these variations during the de-duplication process and often manual review is essential to confirm results.

Cross-language studies - dealing with studies published in different languages introduces challenges due to variations in titles, authors, and other bibliographic details. Non-journal sources - systematic reviews may include various types of sources beyond journal articles *e.g.*, reports, theses, and conference papers. These sources may have different indexing and citation formats, making de-duplication more complex.

Multiple versions of the same studies also contribute to the problem. Studies may be published in different versions (*e.g.*, conference abstracts, and full-text articles) or have been published in multiple journals. Deciding whether these are duplicates or unique records requires careful assessment as "there is currently no standard methodological approach to deal with overlap in primary studies across reviews"[27].

Risk of exclusion - overly aggressive de-duplication can lead to the inadvertent exclusion of potentially relevant studies, affecting the comprehensiveness of the review. Technological limitations - automated tools may not be able to handle certain complexities, such as very similar studies with nuanced differences *e.g.*, differences in journal names "and" instead of "&" or author information or order of authors names[17].

Resource constraints - limited access to paid-for automated tools, lack of personnel, or time can impact the thoroughness of the de-duplication process.

Striking a balance between efficiency and accuracy is essential to overcome these limitations. Navigating these challenges requires a combination of methodological rigor, technological tools, collaboration among the review team, and transparent reporting of the de-duplication process and outcomes.

THE ROLE OF PROSPECTIVE REGISTRATION OF SYSTEMATIC REVIEWS AND META-ANALYSIS AND HOW THIS HELPS IN DE-DUPLICATION?

Prospective registration involves registering systematic reviews and meta-analyses in publicly accessible databases before starting the research process. This practice has gained prominence in recent years, primarily due to its significant impact on de-duplication efforts. In a 2022 paper by a German team from Brandenburg Medical School (Theodor Fontane)[29], the authors stated that prospective registration of systematic reviews aims to reduce bias in research conduct and reporting, increase transparency, and prevent unintended duplication, thereby reducing research waste. There are several options available for prospective registration, including PROSPERO, the Registry of Systematic Reviews/Meta-Analyses in Research Registry, INPLASY, the Open Science Framework Registries, and protocols.io. These registries provide search functions to help authors avoid duplicate reviews.

Prospective registration discourages the submission of the same systematic review or meta-analysis to multiple journals, as researchers and publishers can easily identify prior registrations. Hence reduces the chances of duplicate publications, a common issue in medical literature, which can subsequently lead to de-duplication problems. Registered systematic reviews and meta-analyses are required to provide a detailed protocol outlining their research objectives, methodologies, and inclusion criteria. This transparency helps researchers identify potentially duplicate records, even before data collection begins. Prospective registration fosters collaboration by allowing other researchers to see that other reviews are ongoing or coming up in relation to their own field. But also fosters group work and discourages the chances of having redundant reviews at the same time.

THE ROLE OF REFERENCE CITATION ANALYSIS FOR PROPER CITATION AND DE-DUPLICATION

Another important tool to improve de-duplication in medical databases is reference citation analysis and this goes hand in hand with prospective registration. "Use of the unique registration number may be useful in helping track subsequent use or citation of the review to monitor its impact"[30]. It involves a meticulous examination of the references cited in articles, and it plays a critical role as *via* reference citation analysis, researchers can identify secondary publications that stem from the same primary research, such as conference abstracts, journal articles, and systematic reviews. This is crucial for de-duplication, as it helps consolidate related information into a single reference. Citation analysis also aids in ensuring that the primary sources are correctly attributed and cited in systematic reviews and meta-analyses. But also, can reveal citation errors, discrepancies, or inconsistencies in systematic reviews and meta-analyses. Identifying and rectifying these issues contribute to the overall quality of the research synthesis. This helps maintain accuracy and integrity in the research synthesis process.

BEST PRACTICES FOR DE-DUPLICATION IN LITERATURE REVIEWS

De-duplicating search results and studies effectively during the systematic review process is essential. A comprehensive understanding of the data set's characteristics and proper validation of de-duplication outcomes are also critical. Transparent documentation of de-duplication procedures and reproducibility of results are important. Yet there are no standardized guidelines for all aspects of de-duplication, leading to variations in practices and interpretations as shown in this opinion literature review.

Following best practices helps maintain the quality of the review and the credibility of its findings. The practice proposed in this opinion review reflects the personal approach of the librarian but can be applied broadly to all researchers working on a systematic review to achieve reliable and reproducible results.

Document the process - clearly document your de-duplication process in the review protocol. This documentation should include the criteria for identifying duplicates, the tools/software used, and any decisions made during the process of de-duplication so it could be replicated by others. Transparency in the methodology enhances the credibility of the review.

Utilize reference management software (*e.g.*, EndNote, Zotero, Mendeley) to manage and organize search results. These tools include automated (default) de-duplication features that help identify at least the exact matches and reduce obvious duplicates. *e.g.*, in EndNote the default settings are author, year, and title. These tools can also further help identify duplicates based on predefined criteria *e.g.*, volume, issue, and pages which require deciding on a method that is best for that project *e.g.*, the Bramer method for EndNote.

Manual review - conduct a manual review of potential duplicates identified by some of the automated tools. Establish criteria for matching - define explicit criteria for matching *e.g.*, titles, authors, publication dates, and other bibliographic information to confirm whether records are indeed duplicates. Decide on a threshold for matching to avoid excluding potentially relevant studies.

Handle multiple versions of the same paper - pay attention to different versions *e.g.*, conference abstracts, and full-text articles. Decide whether to treat them as separate records from the start or duplicates based on their content (usually screening in full text will solve this problem).

Address non-journal sources - be prepared to de-duplicate various types of sources beyond journal articles, such as conference proceedings, reports, and theses. Consider their unique indexing and citation formats.

Handle updates and overlapping searches of the existing systematic review strategy. If your review involves multiple search rounds, use the de-duplication process to identify studies already included in previous rounds to avoid the common assumption that updating the search strategy is as easy as taking it from where you left off.

Resolve discrepancies - in case of discrepancies or uncertainties, consult with your review team to make informed decisions about the status of potentially duplicated records. Document decisions - document all decisions made during the de-duplication process, including the rationale for excluding or retaining records. Transparency in decision-making enhances the review's reproducibility.

Remember that while automated tools can expedite the de-duplication process, often manual review is unavoidable and still crucial for accurate identification of duplicates, especially when titles and abstracts are not sufficient for differentiation. Consistency, thoroughness, and transparency are key principles when de-duplicating studies in systematic reviews.

ETHICAL CONSIDERATIONS IN DE-DUPLICATION

While de-duplication is essential for data integrity and research quality, it's important to approach the task in a manner that respects the rights of authors and researchers, maintains data privacy, and adheres to ethical standards *e.g.*, the European Code of Conduct of the Research Integrity published by All European Academies[31]. Ethical considerations in de-duplication align with principles of reliability, honesty, respect, and accountability in responsible research and data management outlined by the above code.

With that in heart, this opinion review proposes the following considerations, transparency and documentation: (1) Systematic review protocol should provide transparent information about the de-duplication process, including the criteria used, methods applied, and decisions made. Transparent documentation helps ensure accountability and reproducibility, allowing others to understand and verify the process; (2) Preservation of data integrity: While removing duplicates is necessary, ensure that the process does not alter or compromise the integrity of the original data. Keep the original data as a reference for any future inquiries; (3) Conflict resolution: In cases of disagreements or uncertainties about the status of a record, aim for consensus within the review team to resolve conflicts ethically and responsibly; and (4) Maintaining original records: Keep a copy of the original duplicated records, even if they are removed from the final dataset. This preserves a historical record of the research process.

FUTURE DIRECTIONS AND CHALLENGES

The future of de-duplication holds exciting possibilities as technology continues to evolve. Continued advancements in machine learning, deep learning, and natural language processing will enable more accurate de-duplication. Deduklick is already on that path as its first de-duplication tool to ease the de-duplication burden. Standardization efforts to harmonize data formats, identifiers, and metadata across different sources would also simplify de-duplication processes. Collaboration across disciplines, ongoing research, and innovative solutions will even further shape the future of de-duplication.

CONCLUSION

Accurate and reliable de-duplication stands as a cornerstone for quality in evidence-based literature reviews. By addressing issues of duplicate records and data redundancies, de-duplication plays a critical role in upholding the scientific rigor, transparency, and overall quality of systematic reviews, making them more trustworthy and impactful resources for evidence-based decision-making. Although this functionality is available *via* many tools not all of them keep up with current advancements in the field of computer science and continue to see de-duplication only as one of many functions' tools were designed to perform. With Deduklick and AsySD the future of de-duplication holds promise for more accurate, efficient methods that can handle increasingly complex and diverse datasets.

FOOTNOTES

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Country/Territory of origin: Italy

ORCID number: Barbara Hammer 0009-0001-6361-0880; Elettra Virgili 0009-0000-9108-9570; Federico Bilotta 0000-0003-2496-6646.

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REVIEW

Crohn's disease and clinical management today: How it does?

Ronaldo Teixeira da Silva Júnior, Jonathan Santos Apolonio, Jessica Oliveira de Souza Nascimento, Bruna Teixeira da Costa, Luciano Hasimoto Malheiro, Marcel Silva Luz, Lorena Sousa de Carvalho, Cleiton da Silva Santos, Fabrício Freire de Melo

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Ronaldo Teixeira da Silva Júnior, Jonathan Santos Apolonio, Jessica Oliveira de Souza Nascimento, Bruna Teixeira da Costa, Luciano Hasimoto Malheiro, Marcel Silva Luz, Lorena Sousa de Carvalho, Cleiton da Silva Santos, Fabrício Freire de Melo, Instituto Multidisciplinar em Saúde, Universidade Federal da Bahia, Vitória da Conquista 45029-094, Bahia, Brazil

Corresponding author: Fabrício Freire de Melo, PhD, Professor, Instituto Multidisciplinar em Saúde, Universidade Federal da Bahia, Rua Hormínio Barros, 58, Candeias, Vitória da Conquista 45029-094, Bahia, Brazil. freiremelo@yahoo.com.br

Abstract

Crohn's Disease (CD) is an Inflammatory Bowel Disease and is characterized by an immune-mediated nature. Its etiology results from the interaction between genetic, enviromental and microbial factors. Regarding pathophysiology, it involves high levels of interleukin (IL)-12, IL-17, and Th1 profile, along with loss of tolerance mechanisms, an increase in pro-inflammatory interleukins, beyond the possibility to affect any part of the gastrointestinal tract. Its symptoms include abdominal pain, chronic diarrhea, weight loss, anorexia, and fatigue, as well as blood in the stool or rectum. Additionally, conditions comprising musculo-skeletal, cutaneous, ocular, hepatic, and hematological alterations may be associated with this scenario and extra-intestinal presentation, such as erythema nodosum, anterior uveitis, osteoporosis, and arthritis can also occur. Today, clinical history, exams as fecal calprotectin, ileocolonocopy, and capsule endoscopy can be performed in the diagnosis investigation, along with treatments to induce and maintain remission. In this sense, anti-inflammatory drugs, such as corticosteroids, immunomodulators, and biological agents, as well as surgery and non-pharmacological interventions plays a role in its therapy. The aim of this review is to bring more current evidence to clinical management of CD, as well as to briefly discuss aspects of its pathophysiology, surveillance, and associated disorders.

Key Words: Crohn's disease; Inflammatory bowel diseases; Diagnosis; Treatment; Immunomodulation; Biological agents

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Core Tip: Today, the clinical management of Crohn's disease (CD) involves both non-pharmacological and pharmacological therapies with the primary objective of inducing and maintaining remission. In this context, anti-inflammatory drugs, including corticosteroids, immunomodulators, and biological agents, can be employed either as monotherapy or in combination. Surgical treatment, while considered palliative, is not curative. Therefore, this review aims to provide an overview of current evidence regarding interventions for CD.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic condition characterized by inflammation in the gastrointestinal tract (GI) [1-3]. It encompasses a variety of diseases, with Crohn's disease (CD) and ulcerative colitis (UC) being the primary recognized types[2,3]. These diseases are persistent, debilitating, recurrent, and immune-mediated, affecting the digestive system[4,5]. The etiology of CD is multifactorial and results from the interaction between genetic and environmental factors and microbial exposure[6]. Several genes have been associated with CD, with the most evident link being related to the NOD2/CARD15 gene, which is associated with an earlier onset of the disease and a family history of CD[7]. Environmental risk factors include smoking, infections (especially *Clostridium difficile*, linked to disease relapses), use of medications (for example, antibiotics, mainly in the first year of life, aspirin, non-steroidal anti-inflammatory drugs, and oral contraceptives), a low-fiber diet, and stress[8]. Currently, the incidence of IBD is notably increasing, especially in developing countries and recently industrialized nations[9]. The disease, previously considered predominantly an affliction of young adults, is now diagnosed across all age groups, with about 25% of patients identified before the age of 20. The peak occurrence in childhood is during adolescence, but approximately 20% of children develop it before the age of 10, and about 5% before the age of 5[10]. Epidemiological studies in Japan also indicate an increase in IBD incidence, especially in males[11]. In Brazil, it is considered to have low rates of IBD. However, there are indications of an increase in its occurrence, even in the absence of detailed information on new cases[12]. Regarding age, research indicates a notable prevalence in individuals between 20 and 50 years old. Age groups between 20 and 60 years exhibited the highest rates of disease, with women registering a higher incidence in both CD and UC[13]. In the context of IBD, inflammation of the intestinal mucosa triggers symptoms such as abdominal pain, diarrhea, presence of blood in stools, weight loss, and the infiltration of immune cells like neutrophils and macrophages that release inflammatory substances, enzymes, and free radicals, contributing to lesions and ulcerations[2]. CD causes segmental inflammation that can affect any part of the digestive system, from the mouth to the anus. It often results in deeper ulcers that traverse all intestinal layers, potentially leading to the formation of fistulas and associated complications[5]. Although it most commonly affects the gut, this inflammatory condition can impact multiple organs[14,15]. The frequency of extra-intestinal manifestations ranges from 6% to 47%[16] and includes joint, mucocutaneous, hepatopancreatobiliary, and ocular manifestations[17,18]. The impact of IBD on patients' quality of life is significant, affecting physical and mental health as well as work performance. Moreover, it imposes a substantial burden on healthcare systems due to its chronic and recurrent nature. If inflammation is not properly controlled, serious complications can arise, such as abdominal abscesses, strictures, and intestinal obstructions, increasing the risk of developing tumors in the GI tract[5]. Therefore, this review aims to describe the current clinical management of CD to assist healthcare professionals in providing adequate care for these patients.

METHODOLOGY

For this review, the authors surveyed relevant articles in the United States National Library of Medicine (PubMed). The descriptors used, along with Boolean descriptors AND/OR, were: CD; pathophysiology; immunology; diagnosis; clinical management; pharmacological; non-pharmacological; aminosalicylates; corticosteroids; immunomodulators; infliximab; adalimumab; vedolizumab; ustekinumab; surgery. The eligibility criteria for this review were based on the discussion of clinical management, covering topics from the diagnosis of CD to the exploration of new therapies. Articles published within the last 10 years and available in English, Portuguese, or Spanish were considered. A total of 24638 articles were initially identified in the database, of which 124 met the inclusion criteria. Articles that did not address the topics mentioned in the title/abstract or upon full-text examination were excluded. Additionally, a manual search of the references in the included articles was conducted, leading to the inclusion of 18 more articles. In total, 142 articles were included in this review. The summary of the articles selection process is shown in Figure 1.



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Figure 1 Summary of the articles selection process.

PATHOPHYSIOLOGY AND CROHN'S DISEASE IMMUNOLOGY

Both immunological mechanisms of innate and adaptive immunity are interconnected on pathophysiology of CD. There is a dysregulation of the inflammatory and anti-inflammatory processes, as well as excessive release of cytokines[19] .

Histologically, the mucosa of the GI tract comprises three types of cells: Goblet cells, Paneth cells, and immune system cells[20]. These cells are crucial for maintaining the structural integrity and defense of the intestines and play an active role in homeostasis[21,22], causing any intestinal injury to potentially disrupt physiological functioning mechanisms, resulting in harmful responses to the body concerning food absorption and digestion[23]. Additionally, when there is tissue damage in the intestine, alterations in the microbiota can lead to mucosal inflammation, recruiting natural killer (NK) cells and monocytes to combat the injury or pathogen[23].

Studies in mice infected with microorganisms have demonstrated the crucial role of innate lymphoid cells (ILC3) in maintaining the integrity of intestinal mucosal tissues. The absence of these cells, along with IL-22, has been linked to the development of IBD[24]. In addition, Th2 profile response is observed in patients with UC while a Th1 profile response in patients with CD[24]. The high levels of interleukin (IL)-12 in the serum stimulate the maturation of immature T lymphocytes to Th1 profile[25]. Subsequently, cytokines released into the intestinal lumen contribute to the chemotaxis of Th1 cells to the mucosa of this organ. Lymphocytes synthesize chemokines like Interferon (IFN)-γ, IL-2, and tumor necrosis factor-alpha (TNF-α), which are related to the migration of neutrophils and macrophages to the site of inflammation, causing further damage to the mucosal epithelium[19,26].

Within this context, other cytokines such as IL-1, IL-6, IL-23, and transforming growth factor-beta (TGF-β) contribute to the differentiation of a Th17 profile during the antigen presentation process among a subset of immature lymphocytes [27]. These cells are responsible for synthesizing IL-17, which stimulates the migration and activity of neutrophils, thereby promoting a more inflammatory environment, along with T regulatory (T-reg) cells[27,28]. Furthermore, the high levels of IL-17 present in the tissues affected by CD can act on the intestinal epithelium, triggering the release of chemokines that further enhance the chemotaxis of inflammatory agents[28,29]. Macrophages and antigen-presenting cells (APC) also contribute to the synthesis of IL-12, IL-6, TGF-β, and IL-23. Therefore, the inflammatory process is capable of creating a feedback loop, continually stimulating the expression of substrates with Th1 and Th17 profiles[26].

Physiologically, the microbiota assist in the immune tolerance process, which occurs when the body's own antigens are captured by APCs, presented to naïve T lymphocytes, and, due to the heightened expression of immunomodulators such as IL-10 and retinoic acid, the lymphocytes are activated into T-reg cells. Studies have demonstrated that in individuals with CD, there is a loss of this tolerance mechanism and an increase in proinflammatory interleukins[30]. Supporting the theory of immune dysregulation between pro and anti-inflammatory processes, studies have shown that even in the presence of high levels of TGF-β and IL-10, which act as regulators of the immune response, local inflammation persists [27]. Furthermore, a diminished action of IL-10, responsible for regulating IL-23 levels, allows for the elevation of the latter, contributing to the stimulation of the Th17 profile[31].

The continuous and exaggerated inflammation of the intestinal mucosa, along with interactions between substrates and cytokines such as IL-13, IL-17, and TGF-β, can stimulate increased synthesis and deposition of the extracellular matrix, potentially leading to the strictures of the inflamed tissue. Furthermore, the inflammatory process could induce

hyperplasia and hypertrophy of the intestinal muscle, contributing to a stenosis process in the affected region[32].

CD is regarded as a systemic illness due to its potential for complications and the presence of various extraintestinal symptoms[33]. Accordingly, the most widespread disorders associated with this condition comprise musculoskeletal, cutaneous, ocular, hepatic and hematological alterations[34]. Firstly, CD-related arthropathy occurs, apparently, due to genetic predisposition and immune system dysregulation[14]. In this sense, the human leukocyte antigen gene (HLA)-B27 is considered a major factor to the development of arthropathies, regarding genetic susceptibility[35-37]. On the other hand, increasing evidence suggests that it could also be linked to an immune-driven inflammatory reaction, especially related to IL-23, which supports IL-17 production and, consequently, neutrophil recruitment and maintenance of the inflammatory status[38,39].

Regarding cutaneous manifestations, erythema nodosum (ED) and pyoderma gangrenosum (PG) are the most prevalent disorders associated with CD[40]. Apparently, both ED and PG are more frequent in women and patients with other extraintestinal manifestations[41]. Also, a study suggests that the susceptibility to cutaneous manifestations is associated with the TRAF3IP2 gene, which plays a role in IL-17-mediated cellular immune responses[42]. The third most prevalent extraintestinal manifestation of CD involves the eye, *e.g.*, episcleritis, scleritis, and uveitis, with approximately 3%-4% of CD patients affected[43-45]. Overall, the onset and maintenance of the inflammatory process in these structures, as mentioned above, seems to be also related to Th17 cells[46]. As for disease susceptibility, several studies suggests a relationship between HLA-DRB1*0103 and extraintestinal manifestations, including uveitis[47].

In terms of the hepatopancreatobiliary system, Primary Sclerosing Cholangitis (PSC) is considered the most common CD-related disorder[48]. While the pathogenesis of PSC is not yet fully understood, there is growing interest in the role of genetic factors, including certain HLA alleles, in its development. To date, HLA-B8, HLA-DRB1*0301, HLA-DRB3*0101 and HLA-DRB1*0401 are associated with PSC susceptibility[48-50].

CLINICAL MANAGEMENT OF THE CROHN'S DISEASE TODAY: HOW IT DOES?

The diagnosis of CD is complex and is made on the basis of symptomatic findings, physical examination, laboratory and imaging tests.

The role of clinical history and physical examination in CD

In terms of clinical aspects, CD presents diverse manifestations that are related to the intensity of transmural inflammation and its location, which, although it has a higher prevalence in the ileocolic segments, can affect any part of the gastrointestinal tract[51]. Symptoms are usually insidious, but can also develop acutely[52]. In most cases, they occur in young patients and include abdominal pain in the right iliac fossa, chronic diarrhea, weight loss, anorexia and fatigue. In cases that colonic inflammation is present, blood may appear in the stool or rectum[52,53]. In this sense, continuous blood loss and reduced absorption of iron, vitamin B12 and folic acid lead to anemia in 6.2%-73.7% of CD patients. Among these, iron deficiency anemia is the most prevalent[54,55]. It is also important to note the occurrence of extra-intestinal symptoms such as erythema nodosum, anterior uveitis, episcleritis, sclerosing cholangitis, osteoporosis, cholelithiasis, venous thromboembolism, nephrolithiasis, as well as arthritis of large joints or axial arthropathies[52-56]. These manifestations are shown in Figure 2.

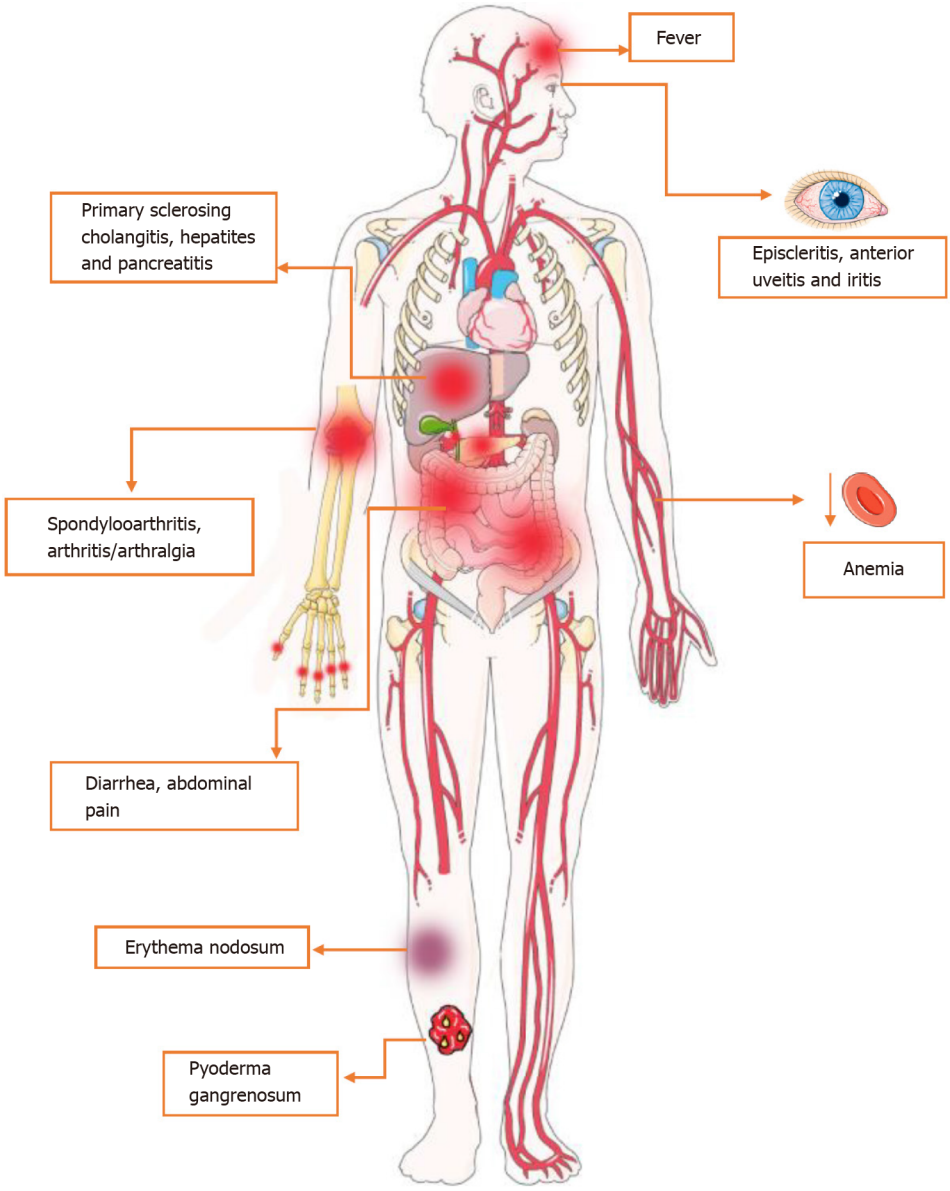
When investigating the patient's clinical history, risk factors such as prior familiar cases of inflammatory bowel disease, diet low in fruit fiber, appendectomy, as well as a lifestyle with poor sleep quality, high stress and little physical activity should be taken into account[53,56]. Among the drugs with potential involvement are antibiotics, oral contraceptives and non-steroidal anti-inflammatory drugs[52,56]. Genetics also seems to be related to CD, among which it can be mentioned that the NOD2 gene in homozygosity increases the risk between 20 and 40 times[52]. In addition, one study suggests that exposure to cigarette smoke, together with chronic obstructive pulmonary disease, leads to systemic and intestinal ischemia, with epithelial dysfunction occurring in the latter and a greater risk of developing more severe forms of CD[57,58]. The physical examination should include an assessment of the hemodynamic state and look for signs of toxemia, malnutrition, dehydration and anemia. The abdomen may show distension or masses. When examining the pelvic and perianal region, lesions on skin or in the anal canal, as well as fistulas with or without abscesses should be investigated [53,57].

How laboratory tests can assist in CD diagnosis

Laboratory tests should be guided by a search for anemia, thrombocytosis, folate and 25-hydroxyvitamin D deficiency and increased acute phase proteins. Fecal Calprotectin (FC), a neutrophil-derived factor for CD in adults with sensitivity 83%-100% and specificity 60%-100%, can be useful for assessing disease activity and monitoring after diagnosis[53,57]. Other potential biomarkers include serum IgA antibodies against *Saccharomyces cerevisiae*[53], antineutrophil cytoplasmic antibodies, antibodies directed against CBir1 and OmpC, elafin for predicting intestinal stenosis in CD[59], as well as microRNA expression screening for intestinal dysbiosis[60,61].

Endoscopic procedures and other imaging exams in CD

Endoscopic procedures are the first line of examination following an assessment of the clinical and laboratory aspects of patients with a non-toxic presentation[52]. Ileocolonoscopy is considered the preferred examination for assessing luminal disease[62], while esophagogastroduodenoscopy is the choice for cases suspected of upper gastrointestinal tract involvement[57,62]. Typical findings may include friability, erosion, segmental inflammation, or aphthoid, longitudinal and serpiginous ulceration. In more advanced cases, it is possible to find fistulas, stenosis, mucosal cobblestoning and



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Figure 2 Clinical presentation that can occur in Crohn's disease patients. The symptoms may be fever, abdominal pain, diarrhea, as well as extra-intestinal symptoms, such as episclerits, anterior uveitis, cholagintis, arthritis, erythema nodosum, and pyoderma gangrenosum.

wall stiffness[56,63]. Additionally, this technique allows for the collection of biopsy samples, characterized by epithelioid granulomas, preservation of globose cells, and transmural inflammatory infiltrate[63]. It is worth noting that endoscopy is also useful for determining the prognosis of CD, as well as screening for cancer associated with colitis and its characteristic lesions[64,65].

One of the limitations of traditional endoscopic methods is the difficulty of accessing the small bowel[66]. Thus, in cases of negative endoscopy, but significant symptomatology and suspicion of small bowel involvement, small bowel capsule endoscopy can be performed, which has a high negative predictive value[53]. The advantages of this method include no radiation exposure, no need for sedation and no pain. However, it is not possible to obtain a sample for biopsy or perform therapeutic interventions[57].

Radiographic techniques are considered for CD affecting the small intestine[53]. Even plain abdominal X-rays can be useful in visualizing dilation, obstruction, perforation or thickening of the intestinal wall. However, they are gradually being replaced by computed tomography (CT), especially CT-enterography, which, although it requires a high volume of intravenous contrast, has become the preferred exam for investigating the wall thickness and the relationships between the intestinal loops[52,56].

Although Magnetic Resonance Imaging (MRI) is a less available and more expensive exam, MR-enterography is an alternative to CT that allows the assessment of mesenteric vascularization and the presence of penetrating disease without the risk of exposure to ionizing radiation[62]. Another important point is that pelvic MRI is the preferred examination for investigating perianal fistulas or adjacent abscesses[53].

Another possibility is ultrasound (US), which, despite being operator-dependent, is highly available, well tolerated and has a sensitivity and specificity close to that of CT and MRI. Also, the accuracy and quality of US for visualizing the intestine can be increased with the use of oral or intravenous contrast[62]. However, it is of limited use in cases where gas is present, present less sensitive for colonic segments, and unable to assess the retroperitoneum and some areas of the GI tract[62,67].

Therefore, it is possible to classify the CD phenotype according to Montreal classification, based on age to diagnosis, localization of inflammatory lesions, and clinical behavior of the disease[65].This classification is shown in Figure 3.

That said, and despite all the complexity of diagnosing CD, professionals should also be aware of differential diagnoses that include ulcerative colitis, intestinal tuberculosis, eosinophilic gastroenteritis, diverticulitis, inflammation of Meckel's diverticulum, intestinal ischemia, chemotherapy-induced enteritis, the presence of a foreign body, neoplasms and others [66,67].

Medical management of CD: How to induce and maintain disease remission

Although IBD has no known cure to date, early medical intervention in the diagnosis of CD can improve clinical response to treatment, reduce inflammatory biomarkers and increase endoscopic remission rates[68]. In addition, patients treated in the early stages of the disease have fewer complications and need for hospitalizations[69]. The goal of conservative clinical management is to induce and maintain remission in patients with active disease, undergoing non-pharmacological interventions and medications, such as aminosalicylates, corticosteroids, immunomodulators, and immunobiologicals[70,71]. Surgical treatment depends on the presentation of the disease[5].

How non-pharmacological interventions can be used in CD treatment

The management of psychological comorbidities, such as anxiety and depression, can improve the disease status, treatment adherence and the need for high-cost care. Cognitive Behavioral Therapy can reduce rates of these comorbidities and improve the quality of life, both individually and in groups, in adults and adolescent patients[72]. A study showed reduction from 35.7% and 25.0% at baseline to 10.4% and 4.2% after therapy in rates of anxiety and depression, respectively[73]. Meditation and relaxation techniques can improve quality of life and possibly decrease inflammatory activity in IBD[74].

Dietetic interventions can be a viable option due to their low cost, availability, and few adverse effects. Diet can influence the immunological system and inflammatory response, although its interaction with intestinal mucosal defense and inflammatory cells is complex[75]. This approach is particularly considered for patients to avoid the use of steroid-based medications, especially children, in whom steroids may impact growth trajectory[76]. Data has indicated an elevated risk for IBD development in individuals with diets high in fat and meat, in contrast to a lower risk associated with high-fiber foods, fruits, and vegetables. Among the dietary options, Exclusive Enteral Nutrition has demonstrated efficacy in inducing CD remission and is considered the first-line therapy, especially in the pediatric population. However, adherence to this therapy can be a challenge in adults[23,77]. Additionally, the use of probiotics showed no significant effect on the induction or maintenance of CD remission[74].

Exercise has a positive impact on various clinical aspects of IBD, including disease activity, the immune system, quality of life, fatigue, and psychological factors[78]. The anti-inflammatory effects of physical exercise are based on myokines, exercise-specific cytokines that are released by myocytes during muscle contractions[79]. Resistance training is a viable and safe option; however, special attention should be given to patients with active disease, as exercise capacity may be limited during this period[78]. Smoking cessation should be strongly considered for smokers with CD, as it assists in modifying the course of the disease, reducing exacerbations, the need for surgical procedures, and improving the response to immunomodulatory therapy[80,81].

Aminosalicylates, corticosteroids, and immunomodulators: pharmacological options?

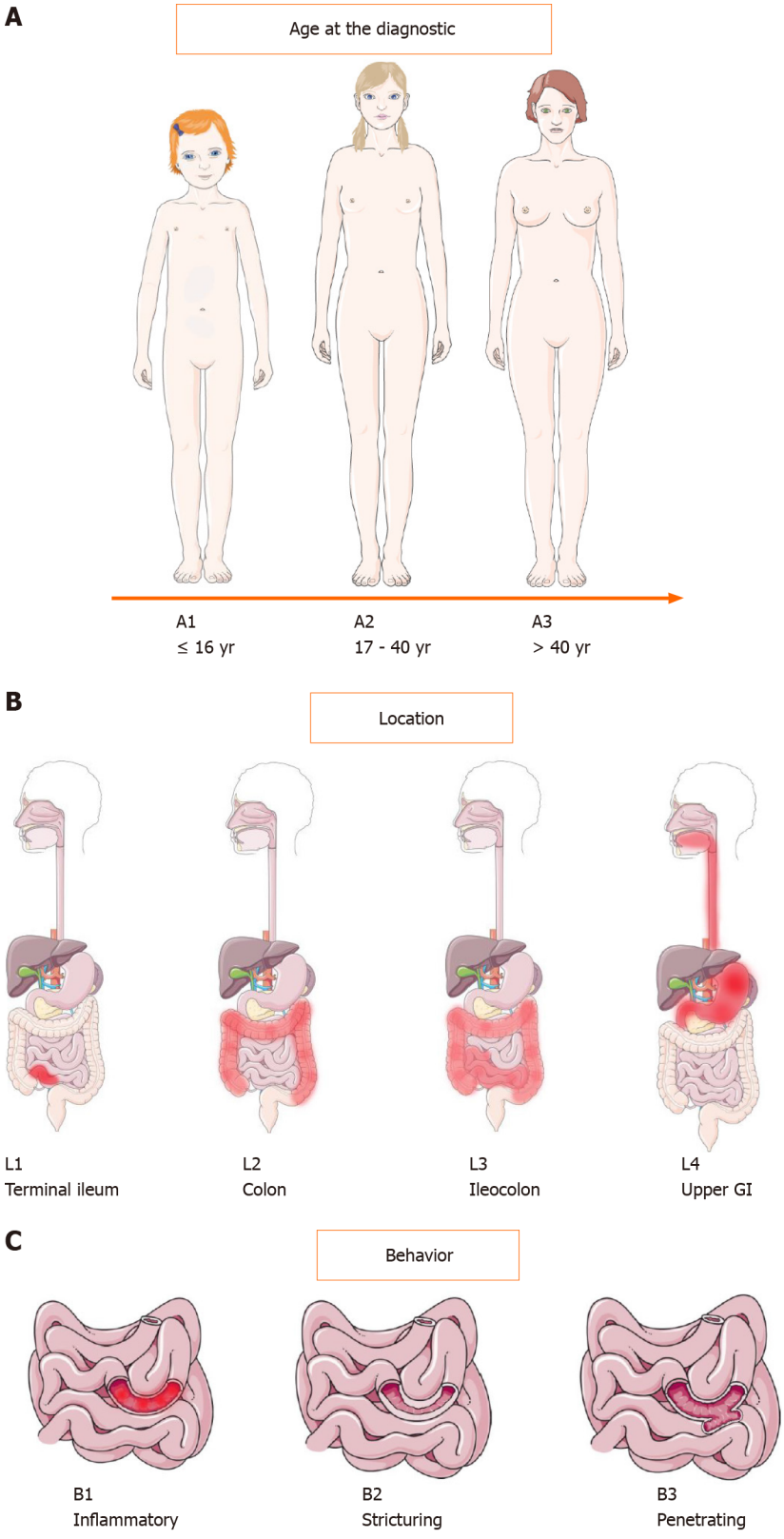
Anti-inflammatory medications are often the first choice in the treatment of IBD, such as corticosteroids and aminosalicylates[82]. Mesalazine, Balsalazide, and Olsalazine are medications derived from 5-aminosalicylic acid (5-ASA). Most guidelines do not recommend 5-ASA for the induction or maintenance period in CD[83,84]. However, 5-ASA is still used by many patients, possibly due to its safety profile, especially in the elderly[85].

Corticosteroids are the first-line treatment at the time of diagnosis and may be indicated regardless of the localization of the inflammatory lesion in moderate or severe disease. Systemic drugs such as Prednisone, Methylprednisolone, Hydrocortisone, and Budesonide can be used, with Budesonide being limited to mild or moderate disease in the ileocecal region[65]. Corticosteroid treatment should be administered for up to 4 wk, followed by a gradual dose reduction until complete cessation of use, typically concluding within 12 wk[86]. According to the British Society of Gastroenterology consensus guidelines, systemic steroids are effective for remission induction but are not suitable for the maintenance phase due to their toxicity and lack of efficacy[87]. For cases resistant to these drugs, immunomodulators or biological therapy may be considered[65].

CD relapse is common after discontinuation of corticosteroid therapy, and immunomodulators such as Azathioprine, Mercaptopurine, or Methotrexate are effective in maintaining remission. Early initiation of these medications is recommended in several cases[87,88]. Methotrexate is not recommended as monotherapy for induction, but it can be used for patients refractory to corticosteroids, with a preference for subcutaneous administration[89].

Biological agents: The role of immunobiologicals in an immune-mediated disease

Immunobiologicals have revolutionized the management of IBD by targeting the inflammatory processes associated with the disease, either by reducing pro-inflammatory cytokines or increasing regulatory cytokines[90,91]. British Society of



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Figure 3 Montreal classification of Crohn's disease. A: It involves age at the diagnostic; B: Inflammation locality; C: Behavior of the disease. For A, ages of ≤ 16 years, 17-40 years and > 40 years are classified to A1, A2 or A3, respectively. Depending on location (B), this disorder can be classified in L1 to terminal ileum, L2 for predominant disease in colon, L3 if ileocolonaccometiment, and L4 for illness in upper gastrointestinal tract. Finally, according to behavior, Crohn's disease can present as inflammatory, stricturing or penetrating, being classified in B1, B2 or B3, respectivelly. GI: Gastrointestinal tract.

Gastroenterology consensus guidelines recommend choosing a biological agent based on clinical factors, cost, safety, availability for use, as well as patient adherence and preference[92]. These drugs include monoclonal antibodies (mAbs) targeting TNFα, such as Infliximab (IFX), Adalimumab (ADA), and Golimumab; mAbs targeting integrins α4β7, such as Vedolizumab; and mAbs targeting the p40 subunit of IL-12 and IL-23, such as Ustekinumab (UST)[82,91].

TNF-α is a critical pro-inflammatory cytokine in intestinal inflammation[92]. Clinical evidence has shown that anti-TNF therapy, such as IFX, leads to better clinical success, early remission, higher rates of mucosal healing, and improved quality of life in approximately 60% of IBD patients[93]. Adalimumab, administered subcutaneously, has also demonstrated efficacy in treating active CD and in patients with a loss of response to or intolerance of IFX[94,95]. However, approximately 40% of patients do not respond to anti-TNF treatment[93].The concomitant use of immunomodulators like Azathioprine or Methotrexate may prevent immunogenicity, reducing the development of anti-drug antibodies and systemic inflammatory status[96].

Various methods are employed to evaluate the response and therapeutic efficacy of these therapies, including biomarkers like FC, C-reactive protein (CRP), serum levels of the anti-TNF agent, and anti-drug antibodies[93]. Reduced levels of FC may precede disease remission and mucosal healing and are correlated with endoscopic scores[97,98]. Also, elevated levels of FC at the onset of treatment may indicate a probable non-response to anti-TNF therapy and lower rates of clinical remission[99,100], and elevated CRP rates are associated with low response in severe CD[101]. Regarding serum levels of mAbs anti-TNF, higher chances of remission and mucosal healing are associated with levels above >2 μg/mL, a minimal concentration for IFX, for example, as well as for ADA[102,103]. Anti-drug antibodies are associated with remission when their levels are below 3.15 U/mL in IFX treatment, while elevated levels may neutralize clinical efficacy, lead to a loss of response, and negatively impact quality of life and disease complications[104,105]. Novel biomarkers, such as proteomic profiles and microRNAs (miRNAs), are still under investigation for clinical practice[93]. Thus, despite the wide availability of biomarkers, most of them are not specific to CD and reflect the body's inflammatory status, necessitating further clinical studies for IBD specifically.

Anti-integrin agents modulate inflammation by targeting integrins, preventing the migration of lymphocytes to the gastrointestinal mucosa. This serves as an alternative to anti-TNF therapy and may be used in patients with a loss of response, inadequate response, and/or anti-TNF intolerance[106]. Vedolizumab, an IgG1 humanized mAbs targeting integrin α4β7 without affecting α4β1, has demonstrated efficacy in inducing remission in CD, with a better safety profile than Natalizumab, another mAbs targeting integrin α4, which is associated with the development of Progressive Multifocal Leukoencephalopathy[106,107].

Ustekinumab targets the p40 subunit of IL-12 and IL-23. Studies have shown its efficacy and safety in the treatment of CD, with approximately half of the patients achieving long-term maintenance of response without loss of response, surgery, or intolerance[108,109]. However, some individuals may experience a loss of response to UST and shorter maintenance times due to factors such as infection, elevated inflammation levels, and inadequate medication concentrations[110,111]. In such cases, optimization strategies can be employed, such as shortening the treatment interval and intravenous reinduction. UNITI studies concluded that the treatment for 12 and 8 wk safely maintained clinical response and remission in patients with CD[112,113].

Surgery in CD: Can be palliative but is not curative

Approximately 15% to 47% of patients undergoing treatment may require surgical intervention[114]. While most patients initially present with the inflammatory phenotype, about 10% exhibit the stenosing phenotype at the time of diagnosis. According to the Montreal classification, within 10 years, the disease progresses to stenosing CD in approximately 15% of cases. An anti-TNF strategy may be considered in cases of strictures without complications, but surgical therapy is indicated for refractory disease[115,116]. In cases of acute small intestinal obstruction, hospitalization and immediate evaluation are necessary to rule out complications such as perforation, abscess, fistulizing disease, and signs of underlying malignancy[115]. Surgical options include segmental resection and stenoplasty[117].

Surgery can provide palliative relief but is not curative. Many patients may experience postoperative recurrence, with risk factors such as active smoking, age younger than 30 years, and previous surgeries for penetrating disease being associated with recurrence[118]. High-risk patients may receive anti-TNF or thiopurine prophylaxis for 8 wk post-surgery, with routine endoscopy, while low-risk patients may undergo endoscopic surveillance 6-12 mo after surgery [119,120]. On the other hand, early resection in CD with ileocecal stricture is associated with prolonged clinical remission and reduced exposure to corticosteroids and biological therapies[121]. Therefore, the decision to undergo surgery should be made on a case-by-case basis, considering disease phenotype and available treatment options.

New therapeutic strategies and patients vigilance

Toll-like receptor 4 (TLR4) is overexpressed in both CD and UC, and the binding between the receptor-ligand pattern recognition of DAMPs or PAMPs triggers a cascade of signaling and recruitment of inflammatory cells and cytokines, such as IL-6 and TNFα[122]. Evidence suggests a potential role for TLR4 antagonists in inflammation treatment, making it a promising alternative for future innovative treatments for IBD[123]. Therefore, more studies are necessary. Additionally, biosimilars are drugs that are similar to immunobiologicals without significant clinical differences in safety and efficacy[82]. The advent of this class of drugs promises to encourage a reduction in the prices of biologics and increase patient access to this form of treatment. Finally, new anti-integrin agents, such as Etrolizumab (humanized mAbs targeting the subunit β7 of the α4β7 and αEβ7 integrins), AJM300 (an orally administered humanized antagonist of α4 integrin), and mAbs IgG2 targeting MAdCAM-1 are in development[106], and more investigations are necessary to establish their efficacy and encourage gastroenterologists to consider them, potentially as first-line therapy in the future.

The vaccination of individuals with IBD varies according to the recommendations of each country, as well as by the clinical judgment of the patient's healthcare provider[124]. It is recommended that these patients be vaccinated before starting immunosuppressive therapy, and if they have already started, vaccines with live viruses should be avoided[125, 126]. Some authors recommend mandatory immunizations for these patients, including vaccines against influenza, chickenpox, hepatitis B, and triple viral. Other authors add to this list the vaccines for hepatitis A, tetanus, diphtheria, whooping cough, herpes zoster, and human papillomavirus (HPV)[127,128]. For hepatitis A and B vaccinations, it is recommended to perform serological tests to evaluate the presence of antibodies and immunity. If the test results are negative or show very low antibody levels, these patients should restart the vaccination schedule with three doses. Additionally, individuals over 50 years old should be immunized against the herpes zoster virus, and persons aged 9 to 45 years should be vaccinated for HPV, preferably covering the four main strains (6, 11, 16, and 18)[126-128]. The pneumococcal vaccine should be administered to all patients, following the recommendations of national immunization programs for chronic diseases[125]. Influenza immunization should be done annually[126]. Furthermore, in the current context, coronavirus disease 2019 (COVID-19) vaccination is essential. Although some studies have demonstrated that seroconversion rates in individuals with CD are similar to those without the disease, others have shown that the level of these antibodies and the duration of this immunity are shorter[129,130]. Therefore, some authors recommend administering one additional dose of the vaccine as a booster approximately 6 mo after completing the vaccination schedule[131].

In general, CD can predispose individuals to various intestinal neoplasms, including colorectal, small intestinal, and anal cancers. Colorectal cancer is the most strongly associated with CD, affecting mainly individuals between 40 and 50 years old who have a history of early onset symptoms[132]. The chronic inflammatory process in the rectal and colonic mucosa, excessive production of free radicals contributing to deoxyribonucleic acid damage, inactivation of tumor suppressor genes, and individual factors are all related to dysplastic mutations and the development of colorectal carcinoma[133]. Additionally, the ongoing inflammation can create an environment favorable to colonization by pathogens like Enterotoxigenic *Bacteroides fragilis*, which has been linked to the development of colorectal neoplasms [134]. Even the use of some immunosuppressive medications, such as TNF inhibitors, has been associated with an increased risk of cancer[135]. Similarly, the inflammatory process in the small intestine, coupled with mutations in genes like TP53 (a tumor suppressor) and IDH1 (which assists in the control of oxidative processes), has also been linked to a higher risk of small bowel adenocarcinoma[136]. Although rare, the inflammatory process of an anal fistula, a complication of CD, can increase the risk of anal cancer[137].

Recommendations for screening for colorectal cancer vary depending on the guidelines of different scientific organizations and local guidelines. Generally, it is recommended to undergo at least one colonoscopy within 8 years of the onset of symptoms to evaluate the extent and degree of mucosal damage[52,138]. TThe American Gastroenterological Association recommends annual screening for high-risk patients, evaluation every 1 to 2 years for individuals with extensive colitis or inflammation located on the left side of the colon, or every 1 to 3 years if two consecutive exams are negative. The management can be individualized based on medical criteria and the extent of mucosal damage[138]. On the other hand, the European Crohn's disease and Colitis Organization recommends annual screening for high-risk patients, evaluation every 2 to 3 years for individuals with moderate-risk factors, and, for low-risk patients, colonoscopy every 5 years[52].

Beyond the considerable physical impact of CD, the disease process can affect various aspects of mental well-being and interpersonal relationships[139]. This disease directly interferes with family planning for couples because, while the pathogenesis of CD is not related to infertility, studies have shown that women with this disease often choose not to become mothers due to conditions such as excessive pain, dyspareunia, anemia, depression, low libido, and malnutrition [140]. Additionally, concerns about the use of medications during pregnancy and their potential side effects, as well as the possibility of active disease during labor, which increases the risk of complications, contribute to reducing discussions about family planning[141]. Other complications such as miscarriages, placental abruption, eclampsia, and pre-eclampsia can be associated with pregnancy and CD[142]. Finally, men using medications or experiencing clinical conditions associated with pain have also been related to voluntary avoidance of having children[140,141].

CONCLUSION

This review provides an overview of the immunologic aspects of CD and discusses the clinical management of these patients. Recognizing gastrointestinal symptoms such as diarrhea, abdominal pain, anorexia, and fatigue can assisti in diagnosis. These symptoms may also be accompanied by extraintestinal manifestations and associated disorders, such as arthropathy, erythema nodosum, episcleritis, and anterior uveitis. Additionally, laboratory tests, such as Fecal Calprotectin, and imaging exams play a crucial role in the evaluation process, with endoscopic procedures being the first-line approach. Both ileocolonoscopy and capsule endoscopy are important tools in this diagnostic scenario. Non-pharmacological and pharmacological treatments form the cornerstone of disease management, with surgical therapy being considered in some cases. This includes the use of anti-inflammatory medications, such as corticosteroids and immunomodulators, as well as biological agents. Therefore, psychological interventions should be more widely prescribed, as they have the potential to improve treatment responses and, consequently, disease outcomes. Finally, therapies targeting the immune system are increasingly being studied, and more research is needed to elucidate the best approach to patients with CD, taking into account their specific phenotypes. Our work summarizes the current evidence available to date.

FOOTNOTES

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Country/Territory of origin: Brazil

ORCID number: Ronaldo Teixeira da Silva Júnior 0000-0003-0835-4092; Jonathan Santos Apolonio 0000-0002-9463-8114; Jessica Oliveira de Souza Nascimento 0000-0002-0332-5228; Bruna Teixeira da Costa 0000-0003-4227-0060; Luciano Hasimoto Malheiro 0000-0001-7767-2874; Marcel Silva Luz 0000-0003-1650-5807; Lorena Sousa de Carvalho 0000-0003-1876-9240; Cleiton da Silva Santos 0000-0002-8261-9338; Fabrício Freire de Melo 0000-0002-5680-2753.

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MINIREVIEWS

Using national census data to facilitate healthcare research

Michael Colwill, Andrew Poullis

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Michael Colwill, Andrew Poullis, Department of Gastroenterology, St George's Hospital London, London SW17 0QT, United Kingdom

Corresponding author: Michael Colwill, BSc, MBBS, MRCP, Doctor, Department of Gastroenterology, St George's Hospital London, Blackshaw Road, London SW17 0QT, United Kingdom. michael.colwill@nhs.net

Abstract

National censuses are conducted at varying intervals across both the developed and developing world and collect detailed data on a wide range of societal, economic and health questions. This immense volume of data has many potential uses in the field of healthcare research and can be utilised either in isolation or in conjunction with other information sources such as hospital records. At a governmental level census data can be used for healthcare service planning by providing accurate population density information but also, through the use of more detailed data collection, by helping to identify high-risk populations that may require increased resource allocation. It can also be a key tool in addressing and improving healthcare inequality and deprivation by both identifying those populations with poorer healthcare outcomes and through helping researchers to better understand the causes of this inequality. Similarly, it has utility when studying the complex causes of disease and assessing the success of strategies designed to tackle these aetiologies. However, the maximum benefit from these various uses can only be realised if the data collection and analysis processes utilised are robust and this requires that census bureaus regularly review and modify their methods in a transparent and thorough way.

Key Words: Census data; Methodology; Epidemiology

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Core Tip: National census data is collected widely across the world. Recently, more detailed data on a wide range of societal, economic and health questions has begun to be collected and this vast volume of data has enormous potential in healthcare research. Examples of potential utility are in assisting with healthcare service planning, analysing healthcare workforces, identifying healthcare inequality and it's causes and understanding the causes of disease. However, census data's utility is dependent upon robust and scientific data collection and analysis and this requires regular methodological review and improvement by national census bureaus.

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INTRODUCTION

National censuses are performed in the majority of countries in the developed world and a growing number of developing countries (see Figure 1). The breadth of the data collected has increased and diversified significantly in recent decades with many countries now collecting data on socioeconomic status and health conditions as well as basic population demographics[1]. Whilst the idea of using census data for healthcare research is not new[2], this vast collection of data remains underutilised. This article will discuss areas in which this data has utility and some of the pitfalls associated with it.

Healthcare service planning

The basic demographic data that is provided by a national census is crucial for all elements of government planning including healthcare provision. At a very basic level, providing accurate data on population density can be used to decide the location and provision of healthcare facilities[3]. An example of this was demonstrated by a Tanzanian study focusing on maternal outcomes in obstetric care based upon proximity to healthcare centres and found that a greater distance to healthcare facilities was associated with worse maternal outcomes[4]. This data has since been used to justify the construction of new healthcare centres in appropriate under-served regions in order to address this disparity.

Some countries go further than just basic population data, such as in the United States where the American Community Survey (ACS) is performed along with the decennial census. The ACS tracks social determinants of health such as income, housing and national origins as well as insurance coverage, fertility and disability. This allows the department of health and human services (HHS) to more precisely target resources to match the anticipated needs of each region and is a key part of HHS' long-term strategy.

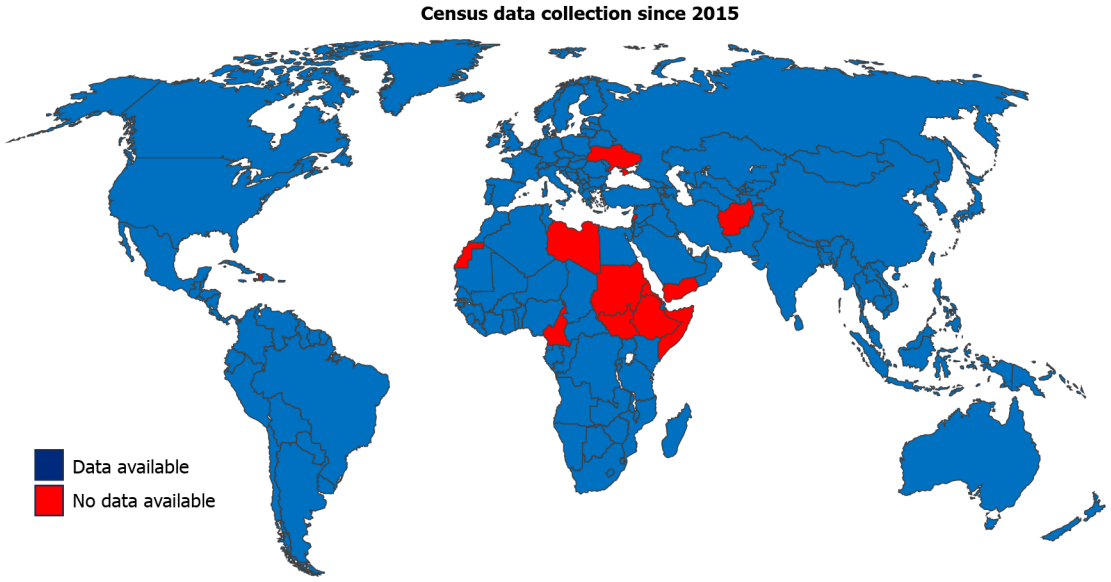
Census data have also been used to analyse the healthcare services themselves as well as the populations they serve. A study from Japan in 2018 used several decades of census data and cross-referenced it with physician surveys and municipality borders to investigate concerns of disparity between the number of physicians in urban and rural practice [5]. The study identified an uneven distribution, which had been worsening over time, with a lack of physicians in rural settings and prompted government departments to start to develop strategies to mitigate this. Workforce analysis was performed in the United Kingdom using census data to review the make-up of the healthcare workforce and identified a heavy dependence upon foreign-trained workers indicating that domestic training programmes needed reforming[6].

Similarly, work by Gupta *et al*[7] used national census data from three different countries to analyse and compare inter-country healthcare worker provision. Their study gave a detailed snapshot of differences between these countries and the various challenges they faced which provided a mandate for the international community, including non-governmental organisations and charities, to direct and focus their resources demonstrating the utility of this data in a transnational, as well as national, setting. It should however also be noted that they found significant variability in the quality of data provided and this imposed limitations on the conclusions they were able to make and this will be discussed later in this article.

Addressing healthcare inequality

Studies have repeatedly shown that healthcare inequality, both at national and international levels, can impact upon mortality and morbidity[8,9] and contribute to deprivation. A recent example was data collected during the coronavirus pandemic which identified differences in outcome with those from deprived health systems having significantly worse survival[10] and thrust the issue of health inequality into the spotlight. Addressing this inequality is a complex political, societal and public health conundrum but census data can be a key element to identify inequalities and guide reform.

In order to allow politicians to appropriately allocate the resources required to address healthcare inequality, the location and nature of the inequality needs to be clearly identified and this is where census data has a role. In the United Kingdom, the 2019 NHS long term plan made addressing healthcare inequality a priority specifically targeting the most deprived 10% of the United Kingdom population. This plan, along with the Core20Plus5 initiative, combined the use of national census data, general practitioner records and hospital records to identify, at a local level, those groups who suffer from the highest levels of healthcare inequality[11]. Some examples of 'at-risk groups' were those from an ethnic minority or those with a disability.



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Figure 1 World map showing which countries and territories have produced national census data since 2015. The figure is created from Powerpoint insert map tool.

Similar use of census data to identify populations with higher risk of deprivation have been used in the United States [12] with some interesting epidemiological findings. One example was the so called 'Hispanic paradox' where historically this population was believed to have better healthcare outcomes despite their high deprivation scores and risk profiles. However, recent and more detailed census data analysis has found that this may not be the case[13] demonstrating the importance of high quality data and statistical analysis.

Census data is also important when monitoring the progress, or lack of, with regards to tackling healthcare inequality. A large study in the United States entitled 'The Public Health Disparities Geocoding Project' used a five step data analysis process to determine, through census and health surveillance data, a picture of health inequalities over time. It identified both areas where improvements had been made but also areas where the problem persisted or had worsened and has been used to inform public policy[14].

Other work has focused on using census data to identify the causes of healthcare inequality. A study in the United States analysed this data and identified a significant association between the presence of greater numbers of liquor stores and the risk of health-related social problems in low income neighbourhoods[15]. Whilst there is clearly not a single cause for poorer health outcomes, this interesting analysis sheds light on possible environmental factors that will be an important part of reducing healthcare inequality.

Understanding the causes of disease

As previously mentioned, census data also have a further role in addressing healthcare inequality by assisting researchers to understand the causes of disease. Canadian researchers, through combining primary care records and census data, demonstrated a link between socioeconomic status and obesity[16] whilst a study in Spain used a similar methodology to demonstrate a link between deprivation and common cancers in order to better target screening programmes[17]. These studies show the utility of census data in assessing health disparities and environmental factors associated with chronic disease.

There are also examples of more detailed and complex use of census data for similar purposes. Moceri *et al*[18] used, in a case-control study, census data and birth certificates to reconstruct the early-life socioeconomic environment of elderly Alzheimer's patients and, through examining variables such as paternal occupation, parental age and birth order amongst others, found higher odds-ratios for developing Alzheimer's for certain characteristics. They also then combined this with genetic analysis of these patients to study the interaction between apolipoprotein ε4 allele and these socioeconomic risk factors.

As well as identifying risk factors for disease, census data have also been used to demonstrate effective interventions in improving public health. Patterson *et al*[19] used census data in England and Wales to demonstrate that active commuting, such as cycling or walking, was associated with lower cardiovascular risk. This is, in theory, a relatively easily achieved public health initiative and there are an increasing number of programmes attempting to increase this method of commuting with the aim of improving public health and reducing the risk of a wide variety of diseases.

Pitfalls

Whilst census data have multiple potential uses there are caveats that need to be addressed and recognised. Firstly, it's utility is dependent upon having robust and accurate data and there have been instances where poor or incorrect collection has had profound social impact. In the United States, the 1840 census incorrectly identified higher levels of insanity amongst the 'coloured' population, an argument then used by slave-owners to suggest that African-American

populations were not able to live as free people[20]. There has also been historically inaccurate data about native American populations and rates of disease leading to worsening healthcare inequality fuelled by the misappropriation of federal funding. A more recent example showed persisting inequalities when it comes to accurate population and health data collection in the Maori population in New Zealand[21] meaning that they receive less resource allocation from government funding. Moreover, there have been documented examples where census data has been deliberately falsified in order to obtain greater funding and support for specific regions. This was discussed and reviewed in detail by Adele with regards to the national census in Nigeria and identified chronic and deliberate falsification of data to obtain benefits from the government[22]. Given the implications for strategic planning and resource allocation, an inaccurate census can have profound impacts for communities and citizens and this is also true when the data is used for healthcare research. These examples underpin the need for a robust scientific process enabling accurate data collection and interpretation in order to have maximum benefit for those who need it the most.

Secondly, there has also been debate around the ethical considerations of using census data for healthcare research. Whilst the data is often anonymised, the nature of census data involves categorisation of respondents and there can be discontent with even simply the names of these categories. A recent example is the controversy surrounding the inclusion of a question asking for respondent's gender identification in England and Wales census for the first time[23]. Similarly, there have been concerns about racial categorisation in association with health labels and stigma[24] although interestingly a public panel consultation in 2018 found that members of the public were in support of census data collection and it's use in healthcare research[25].

Thirdly, there is also a concern that there is inherent bias[26] within the census process itself. This can take the form of non-respondent bias, as described by the United States census bureau[27], which has been shown to skew data significantly such as during the 'poll tax' era in the United Kingdom when non-respondent rates increased. There is also a concern about accurate female representation with this group being historically under-represented[26]. These pitfalls demonstrate the need for regular review of the methodological and analytical practices employed by census bureaus and appropriate improvements if indicated.

CONCLUSION

Internationally, census data collection is becoming more widespread, detailed and robust particularly in the developing world. This data, provided it is accurate to avoid defects, is an immensely rich resource which has utility in research to help with healthcare planning, reducing healthcare inequality and understanding more about the causes of disease. Provided that accurate data is collected in-line with good scientific practice and remains widely and freely available to researchers, it has the ability to be an invaluable resource in healthcare research.

FOOTNOTES

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Country/Territory of origin: United Kingdom

ORCID number: Michael Colwill 0000-0001-6925-8358; Andrew Poullis 0000-0003-0703-0328.

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MINIREVIEWS

Machine learning and deep neural network-based learning in osteoarthritis knee

Harish V K Ratna, Madhan Jeyaraman, Naveen Jeyaraman, Arulkumar Nallakumarasamy, Shilpa Sharma, Manish Khanna, Ashim Gupta

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Harish V K Ratna, Department of Orthopaedics, Rathimed Speciality Hospital, Chennai 600040, Tamil Nadu, India

Madhan Jeyaraman, Naveen Jeyaraman, Arulkumar Nallakumarasamy, Department of Orthopaedics, ACS Medical College and Hospital, Dr MGR Educational and Research Institute, Chennai 600077, Tamil Nadu, India

Madhan Jeyaraman, Ashim Gupta, Department of Orthopaedics, South Texas Orthopaedic Research Institute, Laredo, TX 78045, United States

Shilpa Sharma, Department of Paediatric Surgery, All India Institute of Medical Sciences, New Delhi 110029, India

Manish Khanna, Department of Orthopaedics, Autonomous State Medical College, Ayodhya 224133, Uttar Pradesh, India

Ashim Gupta, Department of Regenerative Medicine, Regenerative Orthopaedics, Noida 201301, Uttar Pradesh, India

Ashim Gupta, Department of Regenerative Medicine, Future Biologics, Lawrenceville, GA 30043, United States

Ashim Gupta, Department of Regenerative Medicine, BioIntegarte, Lawrenceville, GA 30043, United States

Corresponding author: Madhan Jeyaraman, MS, PhD, Assistant Professor, Research Associate, Department of Orthopaedics, ACS Medical College and Hospital, Dr MGR Educational and Research Institute, Chennai 600077, Tamil Nadu, India. madhanjeyaraman@gmail.com

Abstract

Osteoarthritis (OA) of the knee joint is considered the commonest musculoskeletal condition leading to marked disability for patients residing in various regions around the globe. Application of machine learning (ML) in doing research regarding OA has brought about various clinical advances viz, OA being diagnosed at preliminary stages, prediction of chances of development of OA among the population, discovering various phenotypes of OA, calculating the severity in OA structure and also discovering people with slow and fast progression of disease pathology, *etc.* Various publications are available regarding machine

learning methods for the early detection of osteoarthritis. The key features are detected by morphology, molecular architecture, and electrical and mechanical functions. In addition, this particular technique was utilized to assess non-interfering, non-ionizing, and in-vivo techniques using magnetic resonance imaging. ML is being utilized in OA, chiefly with the formulation of large cohorts viz, the OA Initiative, a cohort observational study, the Multi-centre Osteoarthritis Study, an observational, prospective longitudinal study and the Cohort Hip & Cohort Knee, an observational cohort prospective study of both hip and knee OA. Though ML has various contributions and enhancing applications, it remains an imminent field with high potential, also with its limitations. Many more studies are to be carried out to find more about the link between machine learning and knee osteoarthritis, which would help in the improvement of making decisions clinically, and expedite the necessary interventions.

Key Words: Osteoarthritis; Knee; Artificial intelligence; Machine learning; Deep neural network

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Core Tip: Application of machine learning in research has various clinical advances viz, osteoarthritis (OA) knee being diagnosed at preliminary stages, prediction of development of OA, discovering various phenotypes. Large cohorts have been formulated viz, the OA Initiative, the Multi-centre Osteoarthritis Study and the Cohort Hip & Cohort Knee. Many studies are awaited to find about the link between ML and knee OA, which would improve making decisions clinically, and expedite the necessary interventions.

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INTRODUCTION

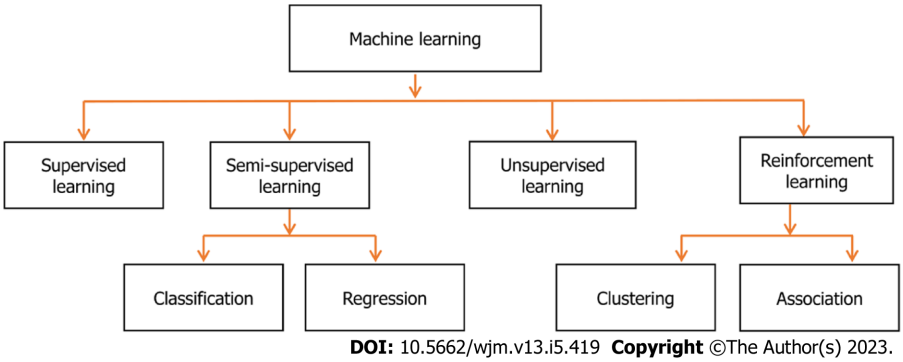
Osteoarthritis (OA) of the knee joint is considered the commonest musculoskeletal condition leading to marked disability for patients residing in various regions around the globe[1,2]. OA is a degenerative disease, still, there is no clear knowledge regarding the factors that are responsible for the progression of the disease pathology. In the late stages of the disease, the only option for treatment is total knee arthroplasty, which is not affordable by many, is very much invasive, and highly degrades the individuals' quality of living.

A breakthrough was brought about by artificial intelligence (AI), specifically by machine learning (ML) in the field of medical care, mainly in subspecialties like rheumatology, mainly OA[3-6]. Machine learning includes Supervised, Semi-supervised, Unsupervised, and Reinforcement learning[7]. Deep learning is a subtype of ML that depends on various layers of a network consisting of a neuron architecture, that allows a model to improvise itself and train on its own, which in turn will lead to great accuracy levels by extracting greater level features from the given data[8-10].

Application of ML in doing research regarding OA has brought about various clinical advances viz, OA being diagnosed at preliminary stages, prediction of chances of development of OA among the population, discovering various phenotypes of OA, calculating the severity in OA structure and also discovering people with slow and fast progression of disease pathology, *etc*. The present models for the prediction of the progression of OA have been based on the collaboration of texture descriptors separated from the trabecular bone, Kellgren Lawrence (KL) grade, and clinical and anthropometric data[11-14]. Methods of Machine learning are divided into supervised and unsupervised (Figure 1). In supervised analysis, the results are established and the data are tagged. Whereas, in unsupervised analysis, the results are not established and the data are not tagged. In addition to these, two new categories were added viz, semi-supervised & reinforcement learning, in which the outcomes are only partially established[7].

Models of semi-supervised learning comprise a mixture of data that are tagged & untagged and are predicated on weak monitoring, with limited tagged data that are used to gain information and for the monitoring of untagged data. On the other hand, reinforcement learning is an ML prototype in which learning happens collectively by a sequence of hit-and-miss trials to increase the result achieved after every hit and thereby increase the learning behavior. Amongst the above-mentioned methods, supervised ML methods are most frequently utilized in the health care systems and medical field[15].

ML is being utilized in OA, chiefly with the formulation of large cohorts viz, the OA Initiative (OAI)[16], a cohort observational study, the Multi-centre Osteoarthritis Study[17], an observational, prospective longitudinal study, and the Cohort Hip & Cohort Knee[18], an observational cohort prospective study of both hip and knee OA. Though ML has various contributions and enhancing applications, it remains an imminent field with high potential, also with its limitations.



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Figure 1 Various algorithms used in machine learning.

LITERATURE REVIEW

Various publications are available regarding machine learning methods for the early detection of osteoarthritis. The detection of osteoarthritis of the knee in its early stages and the exact & persistent assessment needed to detect changes in cartilage were discussed by Mohd Hani *et al*[19]. The key features are detected by morphology, molecular architecture, and electrical and mechanical functions. In addition, this particular technique was utilized to assess non-interfering, non-ionizing, and in-vivo techniques using magnetic resonance imaging (MRI).

Nelson *et al*[20] elaborated on the various causative factors of Osteoarthritis of the knee viz age, mass, and problems to the joint because of the flexing and kneeling activities. The data was attained by the authors from the OAI to assess the advancement of the knee. Dual Echo Steady State MRI was utilized to scrutinize the various images that are available and to choose the part of comparison and later on, perform a computerized differentiation from the data that is available. The modus operandi and design utilized is the Active Appearance Model. The CDI values that are acquired, are fed into machine learning methods like support vector machines (SVM), artificial neural network (ANN) & Random Forest to achieve precise results.

In a study conducted by Kwon *et al*[21], the radiographic maps were used & applied the model of SVM grouping was to authorize the deep learning model. For the multi-classification system, the deep learning network that was used was the Inceptio-ResNet-v2. Calculations of various parameters viz sensitivity, precision, gait analysis, X-ray findings, and F1-values were separated and skilled with property extraction and depth learning features extraction respectively and the training & conditioning of the SVM model was done by utilizing the training set and the compiled data was utilized in the diagnosis of OA. They found an ambiguous learning technique based on radiographic findings, blue-print-dependent scrutiny of data & analysis of functions. Following the inferences of this study improved the accuracy of the diagnosis of OA of the knee.

Wahyuningrum *et al*[22] utilized Long Short-Term Memory (LSTM) for extraction of the features to inquire about the pre-processing through a convolution neural network (CNN). Their main concerns were on the process of cross-validation, of which 2/3 and 1/3 were taken as training and testing data respectively. They performed the analysis by cropping the data of knee images into 400 × 100 pixels which were then arranged as piled augmented images and in turn were introduced in the manner of sequences, as input into the CNN, because LSTM should be performed sequentially. Three convolution filters followed the stride of Max pooling, and 1000 softmax channels into the LSTM processed the entire data from CNN. They have proposed that the LSTM technique is based on the KL grade and is more precise than deep learning.

An analysis of the cases was performed by Galván-Tejada *et al*[23] on the grounds of the data available from the OAI analysis. In this study they utilized two kinds of radiological grades, the first one being a quantitative analysis score and the second being a semi-quantitative analysis score, with the results of radiological images analyzed by two different sets of radiologists. To measure the variables related to pain in the future, A single variable logistic regression test was conducted. They found that the formation of bone osteophytes acts as the predictor of joint pain in the early stages and there's no correlation between pain in the subsequent period and a decrease in joint space. By the findings of the study, they have found that by using plain radiographs the reasons for potential OA knee can be predicted early. They have demonstrated the correlation between the discriminant X-ray of retrogression and the knee pain that developed.

Due to the difficulties faced in diagnosing knee Osteoarthritis, Christodoulou *et al*[24] brought about the concept of deep neural networks for the categorization of issues faced in diagnosis. In this study, to solve the problems in diagnosing osteoarthritis, taking into consideration the variable health factors that cause osteoarthritis, they have introduced a deep learning network to solve the problems faced in classifying knee OA, as a new and efficient machine learning method. The efficiency of the new method was proven by making discrete subgroups of the treated patients, from the data available and by designing a category for the diagnosis of OA knee. Deep neural networks serve as a successful technique in tackling the several problems of machine learning technique viz classification of images, predilection and disease prognosis, *etc*.

Magnetic resonance imaging was utilized by Du *et al*[25] to find the biomedical information concealed in it to diagnose osteoarthritis. Four techniques of machine learning were utilized to assess the osteoarthritis progression and to figure out the transformation in grades according to Kellgren and Lawrence's (KL) classification system, narrowing of joint space in

both medial or lateral compartments. The 36-dimensional elements were divided into two separate 18-dimensional elements with each 18-dimensional element to be individually examined by a four-graded technique for analyzing the various changes of both lateral and medial compartments. In an attempt to narrow down the regions and increase the performance, the analysis by PCA was utilized. By this technique, the machine learning methods for analysis were used to project the increase in KL grades, and medial and lateral compartment joint space reduction, and helped in delineating the pathological progression of knee OA. For the analysis purpose of PCA, the CDI information was compiled from one of the 36-dimensional elements on the 3D knee model of the MR tibiofemoral compartment.

In another study conducted by Kawathekar *et al*[26], they used geometrical parameters from the radiographs of the knee (anteroposterior view), by calculating the distance between the femur and tibia in active form models. Other approaches like local binary arrangement, which is being utilized in most of the pattern recognition tools are also used in the diagnosis of OA knee.

MACHINE LEARNING TECHNIQUES

The typical programming consists of an Algorithm, that includes a couple of rules that require pre-processing data (input) that is utilized to calculate a form of processed data (output) (Figure 2).

The algorithms of machine learning (Figure 3) can learn from the available data and are utilized for the automatic calculation of the set of rules. To perform such approaches, three components[27,28] are essential viz, pre-processed input data, Output data that the algorithm is supposed to predict, and A comparative tool to verify the predicted output performance.

The working mechanism includes entering both the data into the pipeline, which in turn will learn on its own to transform one data into the other data form. Machine learning techniques can be utilized effectively when there is no feasibility or possibility for defining an algorithm by manual techniques and when there's adequate data that applies to the training method.

Various subtypes of machine learning are available like supervised and unsupervised methods. Supervised methods can be utilized when there is a definite idea about the output data and Unsupervised methods can be utilized to discover anonymous data patterns. For instance, Supervised-learning methods encompass linear array regression, gradient boosting and ANN, *etc*.

ARTIFICIAL NEURAL NETWORKS

In the recent past, AI has been used graciously in the field of medicine. AI is nothing but a division of software engineering that includes algorithm training to reflect the human learning process. The design of the human brain has been the basis of various machine learning methods like the ANN. The ANN consists of a congruence of neurons that are interrelated. The ground structure of the neurons, can transform their inner structure or initiate a process, based on the information that is being fed to them and in turn results in a yield that depends upon both the input provides and the present execution.

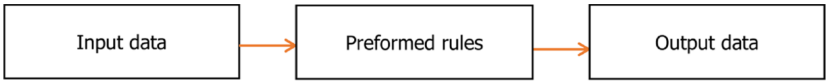
CONVOLUTION NEURAL NETWORKS (CNN)

CNN is utilized widely in various modalities like an assertion of images and videos, recommendation systems, and handling of basic languages. Convolutions are vastly used in processing images[29,30], this being one of the reasons behind its introduction to deep learning methods to perform visual tasks. CNN facilitates the advantage of knowing about the local patterns that are concealed in the data given unlike the other systems, which treat the input data variables globally. CNN belong to a special subgroup of ANN, which uses a system known as a convolution in a minimum of at most one of their steps. Yann LeCunn introduced CNN first in 1990 though it was not that popular at that time[31].

Convolutions involve the mathematical assessment of two different functions that are assertions of real values. In imaging modalities, various terminologies are used to describe the input and kernel, which indicates the first and second functions respectively. The output data that is attained is termed the feature map. CNN models date back to one among the historical deep neural network models, which have various layers that are hidden between the general ones, and this, in turn, helps in knowing more information regarding the features that are concealed in the images that are provided as the input. A second type of layer that is often utilized in CNN models conducts pooling undertaking. Due to this pooling, there is a reduction in spatial resolution and the most appropriate features are only retained, which is solely necessary for maintaining a feasible network size.

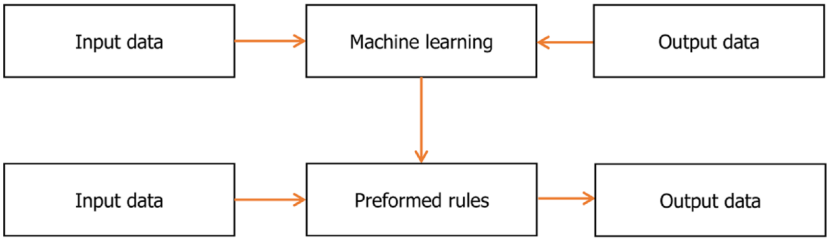
OBJECT CLASS DETECTION

In today's treatment modalities, technologies of machine vision are utilized mostly for the identification of surgical materials among the available objects. The features of medical image inquiry have helped doctors in various ways viz



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Figure 2 Typical programming algorithm.



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Figure 3 Machine learning algorithm.

enhancing the diagnostic capabilities of pediatricians, outcomes which are object-guided, treatment-oriented, and extensive evaluation of various body organs like evaluation of clinical images of those in a state of vulnerability. The availability of advanced technological systems enhances the necessity for identifying and diagnosing various diseases like osteoarthritis, but there are various hurdles to the analysis of its probable location.

Nowadays various algorithms are available which are being used in health care to solve these hurdles. Numerous numbers of various technological advancements are happening in the day-to-day period, which focuses on the diagnosis and recognition of many other disease conditions. The traditional object analyzing methods can be utilized in a broad spectrum of imaging methods viz Radiography, Ultrasonography, Computed Tomographic images, and MRI.

There is no standard method of ML which is acceptable in order to apply them for real-time application[32]. Future research should concentrate on making algorithms that can detect OA in the stages of less severity. The lacunae in the real-time application can be fulfilled by utilizing data in larger numbers and multimodality data to make the ML models more accurate and increase the credibility simultaneously[9].

CONCLUSION

The future of medical science relies upon developing new methods of detecting osteoarthritis in its early stages, which would help in providing joint-preserving treatment modalities to patients. Machine learning techniques would develop a new revolution of augmented radiological methods. Machine learning can explore a wide range of designs, to identify the interaction and multiresolution models, which can easily predict the dynamics of the system to find the predisposing factors. Many more studies are to be carried out to find more about the link between machine learning and knee osteoarthritis, which would help in the improvement of making decisions clinically, and expedite the necessary interventions.

FOOTNOTES

Author contributions: Jeyaraman M contributed to conceptualization; Ratna HVK, Jeyaraman N, and Nallakumarasamy A contributed to initial draft; Ratna HVK and Jeyaraman M contributed to final draft; Sharma S, Khanna M, and Gupta A contributed to supervision; All authors accepted to publish the current version of the manuscript.

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Country/Territory of origin: India

ORCID number: Harish V K Ratna 0000-0002-9144-0612; Madhan Jeyaraman 0000-0002-9045-9493; Naveen Jeyaraman 0000-0002-4362-3326; Arulkumar Nallakumarasamy 0000-0002-2445-2883; Shilpa Sharma 0000-0001-8695-8372; Manish Khanna 0000-0002-2890-869X; Ashim Gupta 0000-0003-1224-2755.

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MINIREVIEWS

Synoptic review on existing and potential sources for bias in dental research methodology with methods on their prevention and remedies

Amit Arvind Agrawal, Nilima Prakash, Mohammad Almagbol, Mohammed Alobaid, Abdullah Alqarni, Hammam Altamni

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Amit Arvind Agrawal, Department of Peridontology and Implantology, Mahatma Gandhi Vidyamandir's Karmaveer Bhausaheb Hiray Dental College and Hospital, Nasik 422003, Maharashtra, India

Nilima Prakash, Department of Oral and Maxillofacial Pathology, Mahatma Gandhi Vidyamandir's Karmaveer Bhausaheb Hiray Dental College and Hospital, Nashik 422003, Maharashtra, India

Mohammad Almagbol, Department of Periodontics Community Dental Sciences, King Khalid University, Abha 62217, Saudi Arabia

Mohammed Alobaid, Department of Restorative Dental Science and Dental Education, King Khalid University, Abha 62217, Saudi Arabia

Abdullah Alqarni, Department of Diagnostics Sciences and Oral Biology, King Khalid University, Abha 62217, Saudi Arabia

Hammam Altamni, Department of Orthodontic, Assir Specialist Dental Center, Abha, Ministry of Health, Abha 62217, Saudi Arabia

Corresponding author: Amit Arvind Agrawal, MDS, MPhil, Doctor, Professor, Department of Peridontology and Implantology, Mahatma Gandhi Vidyamandir's Karmaveer Bhausaheb Hiray Dental College and Hospital, Mumbai-Agra Road, Panchavati, Near Kannamwar Bridge, Nasik 422003, Maharashtra, India. agrodent@rediffmail.com

Abstract

The results of years of dental study serve as the foundation for the practise of medicine and, for that matter, dentistry. Doctors may have their own preferences for techniques and materials, but whether directly or indirectly, their decisions are influenced by systematic reviews and meta-analyses. However, due to poorly conducted or presented research, this very basic foundation may not be reliable. Bias in research is one of several factors that might make study results or research itself unreliable. Bias can be introduced into research at many stages, deliberately or unknowingly. Bias can appear at any point during the research process, even before the study itself begins. There are many biases in research, but some of them are more relevant to dentistry research than others. Because it is said that "eyes

see what the mind knows", it is essential to have a complete understanding of the different types of bias, how and when they get entrenched, and what steps may be taken to prevent or lessen them if they do occur. This comprehensive summary of bias in dentistry research is provided by this synoptic review. The goal is to identify gaps and measures that have been taken-or that should have been taken-by providing both descriptive and evaluative summaries, as well as examples from the literature, when needed.

Key Words: Dental research; Bias (epidemiology); Research methodology; Research design; Epidemiologic methods

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Core Tip: Be it clinical or *in-vitro*, bias may arise at any point in the course of research. Always make efforts to minimise, if not completely eradicate, any potential bias that could show up in a study. However, how can a researcher take preventative or remedial actions if they are oblivious that bias is being introduced into their study? This article lists and summarises every potential bias that could arise during a study so that the researcher is aware of the possibilities and can take the necessary precautions to contribute reliable scientific data to the literature.

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INTRODUCTION

The practice of medicine, or for that matter dentistry, is primarily based on outcomes generated from years of relevant research. Although doctors do have their own preference with regard to procedures and materials, the choice, either directly or indirectly, is based on systematic reviews and meta-analysis. However, this very fundamental base may not be reliable owing to improperly executed or improperly presented research. Of the various reasons that make the research outcome or the research itself unreliable, bias is an important aspect that is either knowingly or unknowingly introduced at various steps.

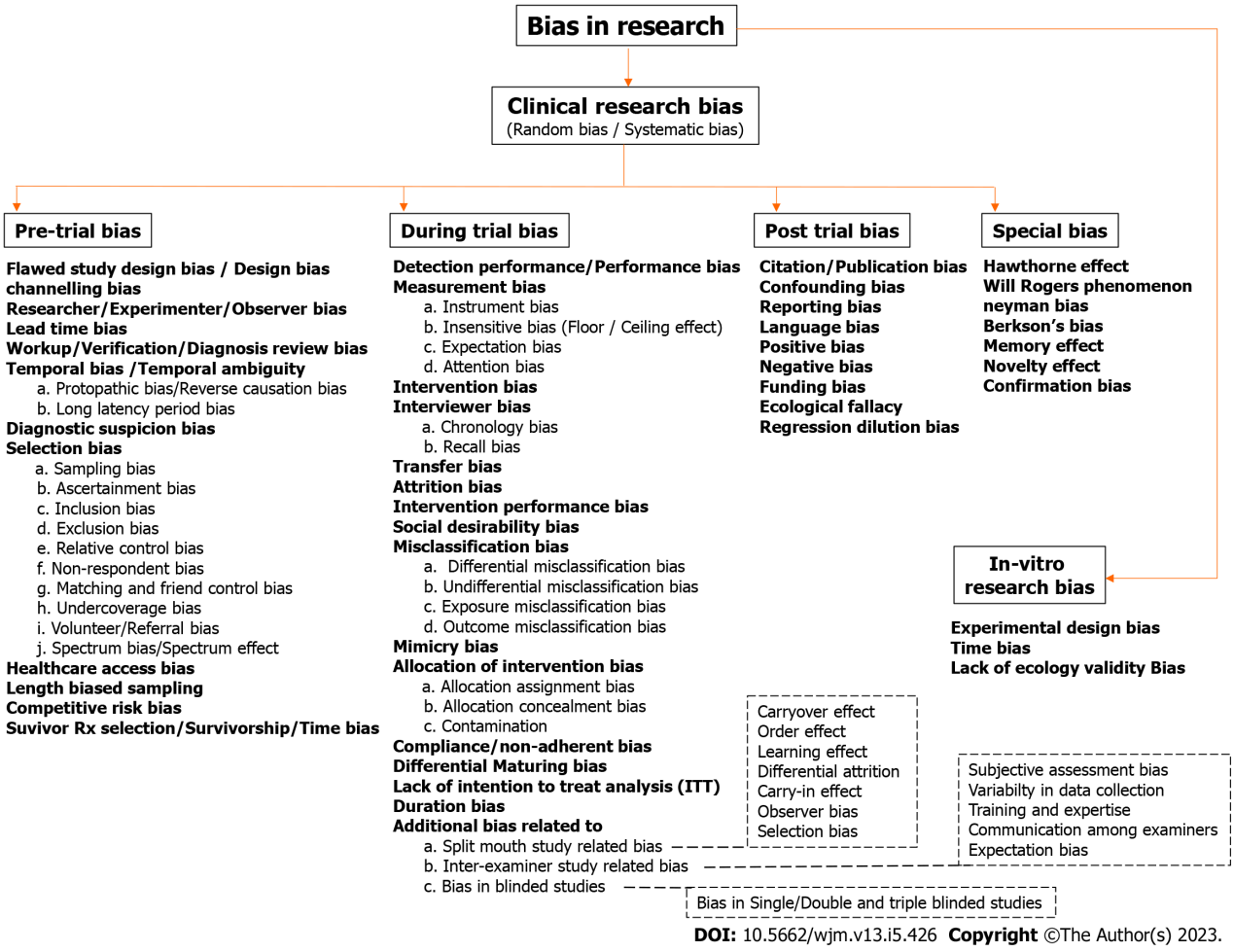
By definition, bias is any tendency that prevents unprejudiced consideration of a question[1]. Bias can occur at any phase of research, even before starting the study itself. Bias is not just a yes or no variable, and its interpretation cannot be restricted to whether bias is present. Instead, a critical reader should consider the efforts taken in a study to decrease the degree of bias and also critically evaluate the influence of the bias on the conclusion[2]. There is a long list of biases in research, but some of them are more applicable to dental research. The "eyes see what the mind knows"; hence, a thorough knowledge of types of biases, how and at which stage they are introduced, and the ways to avoid or reduce them are crucial for justifying the results of the research. Awareness of these biases is imperative to promote rigorous and unbiased dental research. Researchers, peer reviewers, journal editors, and funding agencies should collaborate to establish robust methodologies, transparent reporting standards, and effective peer-review processes to minimize the impact of bias and enhance the reliability and validity of dental research. Additionally, promoting open access to research findings and fostering a culture of transparency and reproducibility can ensure a comprehensive and unbiased body of dental literature.

This synoptic review provides a concise but accurate overview of all materials related to bias in dental research. The aim is to provide both descriptive and evaluative summary along with examples from literature to identify gaps and measures taken or should have been taken. In dental research, like any other field, various types of biases may affect the validity and reliability of the results. A detailed and well-categorized flowchart listing all possible biases that can occur in clinical and *in vitro* research is depicted in Figure 1. A special emphasis is given to certain categories of biases, such as those related to split-mouth study, inter-examiner studies, and blinded studies. Finally, a brief description of various biases that are difficult to categorize under a single category are mentioned in detail under the special bias category. The following detailed discussion about various biases adheres to the headings and subheadings with reference to Figure 1 for ease of understanding and clarification.

BASIC TYPES OF BIAS[3]

Random error/random variability

It refers to the unavoidable fluctuation in data that results from chance. There is no way to completely prevent or control this type of inaccuracy. Measurement precision and accuracy, as well as the outcomes of statistical analysis, can be impacted by random error. To lessen the effects of random bias, researchers often use statistical techniques and large sample sizes. In addition, if investigators are themselves involved in sample collection, it would further reduce the



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Figure 1 An exhaustive list of possible biases that can occur in clinical and *in-vitro* research.

chances of random bias.

Systemic bias (or systematic bias)

It is a type of prejudice that is present throughout the planning, execution, or analysis of research investigations. This bias is systematic or persistent, which means that it does not result from chance but rather from certain defects or problems in the study process. Systematic bias has an enormous effect on the reliability and validity of research findings. Typical examples of systemic bias in research include selection bias, sampling bias, measurement bias, and funding bias. The various types of systemic bias have been discussed under different subheadings along with the measures to be taken to avoid or at least reduce them, wherever relevant.

CLINICAL RESEARCH-RELATED BIAS

The pretrial stage ranges from selecting a topic for the research to literature search, preparing a research proposal, and designing a study and even screening tests and patient recruitment. Bias at the stage of study design and patient recruitment can cause fatal flaws in the resultant data, which are almost impossible to compensate during analysis. The following is the list of possible biases that can occur in the pretrial phase of a research.

Flawed study design bias (design bias)

It refers to a type of systematic bias that arises from errors or inadequacies in the planning, structure, or methodology of a research study. This bias can significantly impact the validity and reliability of the findings as it introduces systematic errors that are not due to random chance. Almost none of the studies with this bias get published in reputed journals as they get rejected either at the editor or reviewer level.

Channeling bias

As the name suggests, channeling bias occurs at the stage when patients are channeled to a specific group intentionally. This bias occurs in nonrandomized trials wherein patients' prognostic factors and degree of illness determine the study cohort in which they are placed[4].

Researcher bias/experimenter bias/observer bias

It occurs when researchers' personal beliefs, expectations, or preferences influence the study design, data collection, analysis, or interpretation. This bias can introduce systematic errors and affect the objectivity and integrity of dental research.

Lead time bias

It occurs when a new diagnostic test or screening method is introduced, which leads to the early detection of a disease without improving the patient's overall health or survival. As the new diagnostic/screening test is more sensitive, the disease is detected earlier. The patients appear to have a longer survival time from the time of diagnosis compared with those diagnosed later using the older methods. This time difference creates the illusion that the new test or method improves patient outcomes.

Work-up bias (verification bias/diagnostic review bias)

It is a potential source of bias in diagnostic studies. Study participants may be subjected to different levels of diagnostic work-up based on their initial test results or other factors. For example, individuals with positive initial test results may receive additional more definitive tests or clinical evaluations, whereas those with negative results may not undergo further assessment. Those selected for verification may not be representative of the entire study population, and this differential verification process can lead to an overestimation or underestimation of the test accuracy. These issues can result in biased estimates of the test sensitivity, specificity, positive predictive value, and negative predictive value.

Temporal ambiguity

It refers to a situation wherein it is unclear or ambiguous whether an exposure (such as a treatment or risk factor) occurred before or after the outcome of interest (such as a disease or health event). This ambiguity can introduce a bias and make it challenging to establish a causal relationship between the exposure and the outcome. In retrospective studies, if the timings of the exposure and outcome are not clearly established, it can lead to temporal ambiguity. Even participants or medical records may not provide accurate or detailed information about the exposure and outcome timings, thus making it difficult to determine which occurred first.

Protopathic bias: Also known as reverse causation bias, it occurs in certain epidemiological studies when the symptoms or early manifestations of a disease/condition may lead individuals to modify their behavior or seek medical attention. This behavioral change or medical intervention may include exposure to a particular treatment, medication, or lifestyle modification. Researchers may mistakenly attribute the exposure (*e.g.*, a specific treatment or medication) to be the cause of the outcome (*e.g.*, the development of the disease) when, in fact, the exposure occurred after the initial symptoms or early signs of the disease developed. For example, in a hypothetical study, an association is sought between amoxicillin 500 mg administered to patients experiencing fever (cause) and gastric upset (outcome as a side effect). Some patients having fever may also have undiagnosed or yet-to-manifest gastric upset. After taking amoxicillin for fever, if gastric upset occurs, although it was already present subclinically and only manifested later on, it would be assumed that the drug caused the side effect.

Long latency period bias: Some diseases or health events have long latency periods, meaning there is a substantial delay between the exposure to a risk factor and the development of the outcome. During this extended period, other factors or interventions may come into play, adding complexity to the temporal relationship.

Diagnostic suspicion bias

Healthcare providers or researchers might have preconceived beliefs or suspicions about the likelihood of a particular diagnosis based on the patient's symptoms, medical history, or other factors. These prior beliefs can influence the decisions made during the diagnostic process. Healthcare providers may be more likely to order specific tests related to the suspected diagnosis and may interpret the results with a bias toward confirming that diagnosis.

Selection bias

It occurs during the identification of a study population wherein participants or subjects are not randomly selected, resulting in a nonrepresentative sample. For instance, if certain demographic groups are overrepresented or underrepresented in the study, it can affect the generalizability of the findings. Selection bias arises when participants or study subjects are not adequately representative of the target population. This bias can occur because of inappropriate sampling methods or exclusion criteria, which may limit the generalizability of the study findings to the broader dental patient population. This type of bias is commonly observed in case–control and retrospective cohort studies wherein exposure and outcome would have already occurred at the time of selection[5]. This bias can be reduced by incorporating strict randomization and blinding with best possible efforts. Allocation concealment should be carefully designed and also reported at the time of publishing the findings.

Sampling bias: It is a type of selection bias wherein the sample is not representative of the target population. For example, if the study only includes patients from a specific dental clinic and not from other clinics in the area, the findings may not reflect the characteristics of the broader population.

Ascertainment bias: This bias arises as a result of disproportionate statement of the eligible population. Here, the category of patients examined does not correspond to the incidents in the population.

Inclusion bias: Inclusion bias occurs when study participants are selected to represent the research population, and groups with different experiences are ignored. This bias is especially common in quantitative research. For example, an online oral health survey is supposed to be conducted using available internet sources. One may choose a study group based on possible confounding factors but might skip the fact that some people have access to the internet while others do not.

Exclusion bias: Exclusion bias occurs when some subgroups are intentionally excluded from the sample population before randomizing them into groups. For example, in a periodontal surgery in which patients should be followed up for 4-6 mo, intentionally excluding those patients who stay very far from the research site to avoid later dropouts can result in exclusion bias.

Relative control bias: This type of bias occurs when patients' relatives are included in the study as a control group. This inclusion may or may not have an effect on the actual outcome of the study depending on the parameters to be checked. If it is a placebo-controlled trial, patients and their relatives can easily discuss the intervention received by them and might use each other's medicines.

Nonrespondent bias: This type of bias usually occurs in a large-scale survey, prospective study, or longitudinal study. Many participants may not respond to a survey; however, their actual opinion might be significantly different and would have affected the results of the study if all of them had responded. Maintaining a patient record (email ID/phone number/address) might help in reaching out to non-responders and reducing this bias.

Matching and friend control bias: Friend controls are useful because researchers can quickly locate them *via* the instances. Friends are expected to be more motivated and have higher response rates than general population controls who are unaware of the case. Introverted people will appear on fewer or even no friend rosters, and sociable people will appear on more of those rosters. Extroverts are therefore more prone than introverts to develop into friend controllers. Based on the degree to which the exposures of interest are linked to sociability or personality, selection bias will alter the results[6].

Under-coverage bias: Under-coverage bias arises when a representative sample is drawn from a small proportion of the target population. Online surveys are especially vulnerable to under-coverage bias. For instance, if a survey on oral precancerous lesions is planned and the researcher includes patients visiting a dental hospital as the study population, the vast majority of people who may be at a greater risk but not motivated enough to visit a hospital will be left out. The results of such research cannot be reliably applied to the wider population.

Volunteer/referral bias: Part of the study population who either volunteered for the study or were referred for the specific disease would logically be more motivated for the treatment and follow-ups than non-volunteers. The results of such a study would have very low external validity. For instance, in a study on the prevalence of oral precancerous lesions in young adults, if the study population comprises patients visiting/referred to a dental hospital for consultation, the results would not apply to the general population.

Spectrum bias: Also known as spectrum effect or spectrum of disease bias, it is a type of bias in clinical research and diagnostic testing. This bias occurs when the characteristics of the study population do not accurately represent the broader population of individuals who may be affected by the condition of interest. In other words, the study sample does not adequately capture the entire range or spectrum of disease severity or characteristics seen in the real-world population.

Healthcare access bias

It describes a situation in which the accessibility and availability of healthcare services and resources might have an impact on the demographic characteristics or results of the study. This bias can appear when specific groups of people have unequal access to healthcare services, thereby resulting in the unequal representation of those groups in research studies or in divergent health outcomes.

Lenth-biased sampling

It occurs when the selection of study participants is more likely to include individuals who have experienced a longer duration of disease or exposure. This bias can occur because individuals with longer durations of disease/exposure are more likely to be detected or referred for testing. This bias can result in the overestimation of the sensitivity of a diagnostic test because it is more likely to identify cases in later stages of the disease, which are easier to detect. Conversely, it may result in the underestimation of specificity because individuals with shorter disease durations or milder conditions may not be included.

Competitive risk bias

Competitive risk may cause bias in epidemiology and survival studies. This bias occurs when researchers study time-to-event data without properly considering competing hazards. These are circumstances that could prevent the relevant event from occurring or alter the likelihood of its occurrence. To prevent skewed findings and incorrect conclusions, competitive risk should be considered in the study. Other occurrences that could precede or compete with the main event are referred to as competitive risks. For instance, while evaluating cancer-specific mortality in a cancer study, death from causes unrelated to the disease is regarded as a competitive risk.

Survivor treatment selection bias

A type of bias that can occur in observational studies, particularly in those assessing the efficacy of therapies or interventions, is the survivorship bias or immortal time bias. The bias in survivor treatment selection occurs when individuals who suffer the relevant event (such as death) soon after the start date of the study or before they are eligible to receive the therapy are not included in the analysis. Short-term non-survivors are not included in the study because only people who have survived long enough to receive the treatment are included, which can give the impression that the treatment is more beneficial than it actually is. This bias may cause the advantages of the treatment to be overestimated. The phrase "immortal time" describes the timeframe during which people are regarded as "immortal" because they have not yet gone through the event (such as death) or have not yet received the therapy of interest.

DURING-TRIAL BIAS

Detection performance bias

This type of bias occurs when the measurement or assessment of outcomes is influenced by the researcher's knowledge of the participants' exposure status. This bias happens when there are differences in the care or treatment provided to study groups owing to the knowledge or expectations of healthcare providers or participants. For example, in a clinical trial assessing the effectiveness of a new drug, if the healthcare providers are aware of the treatment assignment (*e.g.*, *via* differences in appearance or side effects), they may provide varying levels of care to participants in the treatment and control groups, thereby leading to performance bias.

Measurement bias

Instrument bias: In many research investigations, data are gathered with the aid of measurements tools, such as questionnaires, scales, sensors, lab equipment, and diagnostic testing. These tools measure or evaluate specific variables of interest. When an instrument consistently overestimates or underestimates the true value of a variable being measured, bias is introduced, which produces unreliable results. The proper selection of a good-quality instrument can reduce this bias.

Insensitive measure bias: Also known as "floor effect" or "ceiling effect", it is a type of measurement bias that can occur in research when a measurement instrument or scale is not sensitive enough to capture the complete range of values or responses within a sample. This bias can lead to inaccurate or incomplete data, thereby affecting the validity and reliability of the study findings. "Floor effect" occurs when a measurement instrument is unable to detect or distinguish values at the lower end of the range. Hence, a substantial portion of participants score at the lowest possible value, creating a "floor" for the data. Conversely, the "ceiling effect" happens when the instrument cannot differentiate values at the upper end of the range. Several participants achieve the highest possible scores, creating a "ceiling" for the data.

Expectation bias: Also known as observer-expectancy bias or experimenter-expectancy effect, it is a type of cognitive bias that can impact the outcomes of studies, particularly experimental and observational research. This bias occurs when researchers, either knowingly or unknowingly, have certain expectations about the results of the study, which can influence their observations and potentially affect participant behavior or data collection. In a study by Nevins *et al*[7], the questionnaire given to the test group was forwarding or positive-answer provoking, which could have obvious answers.

Attention bias: It is type of cognitive bias arising from selectively focusing on certain stimuli or information while ignoring or downplaying others. Individual cognitive processes and experiences may contribute to this bias. Attention bias can influence how participants perceive and react to intervention or stimuli in clinical research. For instance, owing to ingrained beliefs, concerns, or expectations, participants may pay more attention to specific symptoms, treatments, or results. Participants' reports of their experiences may be impacted by attention bias, which could result in inaccurate self-reporting.

Intervention bias

Interviewer bias: It refers to the systematic difference in the manner in which information is solicited, recorded, and interpreted[5,8]. This bias can be minimized or eliminated if interviewers are blinded to the outcome of interest or if the outcome of interest has not yet occurred, as in prospective studies.

Chronology bias: Comparing the intervention with historic findings creates chronology bias. Historic controls are past data rather than present data. Hence, comparing current interventions with such data may affect the results because the two data are collected in different timeframes. Over the years, the methods of diagnosis, investigations, and treatment change, which might improve the results and decrease the rate of complications. For example, a study to identify the prevalence of the MB2 canal (mesio-buccal canal) in upper first molar using cone beam computed tomography (CBCT) and comparing the results with data published decades earlier when CBCT was not available will yield biased results. Such bias can be minimized by conducting prospective studies, or if retrospective, the data should be compared only with findings in the recent past.

Recall bias: In studies that rely on participants' memory or self-reporting, there is a risk of recall bias. Participants may not accurately recall past dental experience or behaviors, thus leading to inaccurate data. This bias occurs when participants in a study inaccurately remember or report past events or experiences. In dental research, this bias can affect

studies that rely on self-reported data, such as surveys or questionnaires, leading to distorted associations between exposures and outcomes. The simplest way to reduce this bias is to frame questions in such a way that it is easy for participants to recall and/or questions from the recent past.

Attrition bias: Also known as dropout bias or nonresponsive bias, it is a type of bias than can occur in research studies when there is a differential loss of participants or data during the course of the study. This bias arises when the characteristics of individuals who dropout or are lost to follow-up are different from those who remain in the study. This can distort the findings and compromise the validity of the results. Attrition bias can manifest in various types of research, including longitudinal studies, clinical trials, surveys, and observational studies. Some common reasons for attrition include participant withdrawal, loss to follow-up, nonresponse to surveys, and incomplete data collection.

Intervention performance bias: It arises when there are differences in the care or treatments provided to different study groups, which can affect the outcomes. For example, if one group receives better dental care than the other, it could influence the results. Similarly, if a particular treatment in a study, such as periodontal surgery or disimpaction, is performed by two or three different doctors, it can result in bias. The variations in the performance of different clinicians might affect the outcomes.

Social desirability bias: Social desirability bias is a form of response bias that is related to systematic errors in the way participants respond to survey questions, interviews, or assessments. To win others' praise or to escape criticism from others, people may deliberately or unconsciously alter their responses to conform to cultural standards, values, or expectations. The responses may not accurately reflect the participants' actual experiences or behaviors owing to social desirability bias. This bias might have an impact on survey results, patient-reported outcomes, self-reported data, *etc.* Aggarwal *et al*[9], in their study on an oral questionnaire about tobacco smoking status, validated the social desirability bias by identifying that a whopping 30% of the participants underreported their smoking status.

Misclassification bias

Differential misclassification bias: Misclassification bias occurs when the exposure or outcome status of the study participants is incorrectly classified. This can happen for various reasons, including measurement errors, inaccurate data collection methods, or imperfect diagnostic tests. Differential misclassification occurs when the probability of misclassification differs between comparison groups in a systematic manner. In this scenario, the bias introduced can lead to incorrect associations that favor one group over another. This type of bias can either exaggerate or attenuate the true effect.

Nondifferential misclassification bias: This bias occurs when misclassification occurs randomly or with equal probability in all study groups. In such instances, the bias introduced may be toward the null (*i.e.*, it tends to make associations appear weaker than they truly are), but it does not systematically favor one group over another.

Exposure misclassification bias: It occurs if the exposure itself is poorly defined or if proxies of exposure are utilized.

Outcome misclassification bias: Here, misclassification of the outcome can occur if nonobjective measures are used to define the outcome. For example, using change in color of the gingival margin to determine the presence of gingivitis instead of a more objective method, such as gingival index or sulcular bleeding index.

Mimicry bias

It occurs during trial when the investigator is examining how exposures are related to a disease. It is important to ensure that the outcome being investigated is the true disease and not a condition mimicking the disease, which could lead to false conclusions about the causes of the disease of interest.

Allocation intervention bias

It typically refers to a type of bias that can occur during the process of assigning the study participants to different intervention groups in experimental research settings. This bias, if not properly managed, can undermine the validity of the study findings and often arises in randomized controlled trials (RCTs) and other experimental designs.

Allocation assignment bias: This occurs when the investigator either consciously or unconsciously assigns certain participants to a specific intervention group based on their characteristics or other factors. For example, if participants who are more likely to benefit from the intervention are placed in the treatment group and those less likely to benefit in the control group, it introduces selection bias.

Allocation concealment bias: This type of bias occurs when the process of randomization itself is not adequately concealed from the researchers or study staff who are responsible for assigning participants to groups. If researchers are aware of upcoming assignments, they may either inadvertently or intentionally influence the allocation process.

Contamination bias: This bias occurs when participants in different intervention groups interact or share information with each other, leading to the exchange of elements of the interventions. This exchange can blur the distinction between groups and undermine the study's ability to determine the true effects of the interventions.

Compliance bias

The degree to which study participants follow the recommended therapy or intervention protocols is referred to as compliance. For instance, participants in clinical trials might be told to take a certain drug, adhere to a certain diet, or make a certain behavioral change. When the participants do not adhere to these guidelines diligently, noncompliance happens. When there are systematically different compliance levels across treatment groups (such as intervention groups) and control groups (such as placebo groups) or between subgroups within the same study group, compliance bias is present. Such discrepancies in compliance may skew the study findings.

Differential maturing bias

This bias is observed in group randomized trials and reflects uneven secular (long-term) trends among the groups in the trial, favoring one condition over another[10].

Lack of intention to treat analysis

Clinical trials and other intervention studies sometimes employ intention to treat (ITT) analysis, wherein all participants are evaluated according to their initial treatment assignment regardless of what transpired later, such as noncompliance, dropouts, or protocol violations. ITT analysis ensures that the groups being compared remain comparable even if some participants did not adhere to their assigned therapy, thus maintaining the advantages of randomization. In the absence of ITT analysis, the research may be systematically different from those who discontinue or do not fully adhere to the treatment, thereby resulting in exaggerated or underexaggerated treatment effects that could mislead clinical or policy decisions.

Duration bias

The length of the study or follow-up period is a crucial factor in clinical research. This period should be selected in accordance with the study question and the desired results. Long-term effects or late-occurring events may not be captured if the study duration is too short. On the contrary, very protracted follow-up times can be expensive and may result in participant attrition, making it difficult to sustain the validity of the study. These issues cause duration bias in either situation. Additional bias related to: Split-mouth study-related bias, inter-examiner-related study bias, bias in blinded studies, questionnaire/survey-related bias.

BIAS ASSOCIATED WITH THE SPLIT-MOUTH STUDY DESIGN

The split-mouth study design is a type of within-subject design often used in clinical research, especially in dentistry and some medical fields. In this design, each subject serves as their control, with one side of the mouth (*e.g.*, left side) receiving one treatment or intervention and the other side (*e.g.*, right side) receiving a different treatment or intervention. Some potential biases related to the split-mouth study design are as follows: (1) Carryover effect: There may be a carryover effect from one treatment to the other, particularly if there is a washout period between interventions. This effect could impact the results of the study as the treatments may not be entirely independent of each other; (2) Order effect: The order in which the treatments are administered can introduce bias. For example, if one treatment is more effective, it might influence the response to the subsequent treatment on the other side of the mouth; (3) Learning effect: Participants may become more accustomed to the study procedures or interventions over time, resulting in different responses for later treatments compared with earlier ones; (4) Differential attrition: There might be differences in dropout rates between the two sides of the mouth, which could introduce bias in the analysis; (5) Carry-in effect: The baseline characteristics of the two sides of the mouth may not be entirely equal, leading to potential confounding; (6) Observer bias: If the outcome measures are assessed subjectively or by different observers, biases may arise because of variations in interpretation or assessment; and (7) Selection bias: The choice of the split-mouth design could introduce bias if certain participants are selected for the study based on specific criteria or characteristics.

To mitigate these biases, researchers employing a split-mouth study design should carefully plan the study, randomize the order of treatments, and ensure standardized and blinded assessments of outcomes. Furthermore, it is essential to consider the potential for bias when interpreting the results of such studies and to be cautious while making generalizations beyond the study population.

INTER-EXAMINER-RELATED STUDY BIAS

Also known as inter-rater or inter-observer bias, it refers to a type of bias that can occur in research studies when different examiners or observers assess the same subjects or data points differently. This bias can influence the results and conclusions of the study, potentially leading to inaccurate or misleading findings. Understanding and addressing this bias is important to ensure the validity and reliability of research outcomes. Some common factors contributing to inter-examiner bias are as follows: (1) Subjectivity in assessments: When examiners employ subjective criteria for evaluating subjects or data, their interpretations and judgments may vary. For instance, in dental research, different doctors may diagnose the same condition differently based on their experiences and personal biases; (2) Variability in data collection: Different examiners may use slightly different methods or instruments to collect data, leading to inconsistencies in the collected data. This variation can affect the reliability of the results; (3) Training and expertise: The level of training and

expertise may differ among examiners, leading to differences in their ability to interpret and analyze the data accurately; (4) Communication among examiners: If there is a lack of standardized communication or guidelines for examiners, they may inadvertently influence each other's judgments, leading to biased outcomes; and (5) Expectation bias: Examiners might be influenced by their expectations or prior beliefs about the study outcomes, consciously or unconsciously affecting their observations and assessments.

Several strategies can be employed to mitigate inter-examiner bias and enhance the reliability of the findings. Implementing clear and standardized protocols for data collection and assessment can reduce variability between examiners. Inter-rater reliability can be calculated to assess the level of agreement among examiners, which can help identify potential bias and guide improvements in the assessment process. Moreover, consistent training can be provided to all examiners to ensure that they have a shared understanding of the assessment criteria and methodologies. In some cases, blinding the examiners to certain aspects of the study (*e.g.*, treatment groups) can help prevent their expectations from influencing the results. In addition, having multiple independent examiners evaluate the same subjects or data can help identify discrepancies and potential biases. A review process can be implemented in which an experienced and impartial researcher checks and validates the assessments of different examiners. By recognizing the potential for inter-examiner bias and taking steps to minimize its impact, researchers can enhance the credibility and accuracy of their findings. One of the best examples of measures taken to reduce inter-examiner bias has been presented in our previous study (Agrawal[11], 2011) on the reliability and reproducibility of a sign grading system for plaque and calculus.

BIAS IN BLINDED STUDIES

Bias in research can occur in various forms, and single, double, or triple-blind studies are not entirely immune to these biases. Although these study designs are adopted to reduce certain types of biases, they may still be affected by other sources of bias. The ways in which each study design can be impacted have been discussed below: (1) Single-blind studies: In a single-blind study, the participants are unaware of their group assignment (*e.g.*, treatment or control), but the researchers are aware of the groups. Bias can still arise in this design if the researchers inadvertently influence the participants' behavior or the study outcome. For example, unintentional cues or communication from researchers to participants may subtly affect the way in which participants respond or behave during the study; (2) Double-blind studies: In a double-blind study, both the participants and the researchers directly involved in the study are unaware of the group assignment. This design helps reduce potential bias caused by participants' expectations or researchers' conscious or unconscious influence. However, bias can still occur if those administering the treatments or interventions (*e.g.*, nurses or doctors) are aware of the group assignment and unconsciously treat participants differently based on that knowledge; and (3) Triple-blind studies: Triple-blind studies go one step further by also keeping the data analysts or statisticians blinded to the group assignment. This design aims to minimize bias during data analysis. However, researchers might still inadvertently introduce bias during the conduct of the study even if the analysts remain blinded to the groups.

POST-TRIAL BIAS

Bias in research after the conclusion of the trial could occur during data analysis or publication or interpretation of the results. Some possible biases that can occur in the post-trail stage are discussed below.

Publication bias/citation bias

This bias occurs when studies with positive or significant results are more likely to be published and those with null or negative findings are less likely to be published. In dental journals, 82% of the published articles report positive results [12]. This bias can create an overrepresentation of certain results and lead to an inaccurate overall picture. It occurs when research findings are selectively published based on their statistical significance or favoring positive outcomes. This bias can lead to an overrepresentation of studies with favorable results, potentially distorting the overall understanding of the effectiveness or safety of dental interventions. Despite the measures adopted by the International Committee of Medical Journal Editors[13,14], citation bias continues to exist. Although centralized documentation of all trials provides information about unpublished trials, the results of such studies can only be speculated. Locating unpublished studies *via* trial registers, search engines, and other databases can reduce this bias.

Confounding bias

It occurs when the observed association between variables are attributed to three possible causes: The exposure itself, the outcome of interest, and an independent factor[5]. One such example is the association between obesity and dental caries (confounded by frequent eating or a high-sugar diet). After the completion of the study, the identified confounders can be controlled by analyzing the association only in those cohorts that are similar in terms of the identified confounding factors. Stratified analysis or multivariable regression analysis can be used to control the identified confounders; however, the role of unidentified confounders cannot be controlled. Unknown confounders can, however, be controlled with randomization.

Reporting bias

It involves the selective reporting of outcomes within a study. Researchers may emphasize certain findings while downplaying or omitting others, which can skew the overall conclusions.

Language bias

This type of bias may occur when research studies published in a particular language are favored over those published in other languages, resulting in an incomplete representation of the available evidence.

Positive bias

The value, effect, or data observed are greater than the actual or casual data, which is away from null. Here, the perceived value is closer to 1.0 than the actual value.

Negative bias

In this case, the value, effect, or data observed are less than the actual/casual data and can be termed null. The perceived value is lower to 1.0 than the actual value.

Funding bias

If the research is funded by a specific dental product manufacturer or entity with vested interests, there might be a tendency to favorably report outcomes related to their products. The bias refers to the potential influence of financial or nonfinancial conflicts of interest on the study outcomes. Dental research studies funded by industry sources might have a higher likelihood of reporting results favorable to the sponsor's products or interests, which can compromise the impartiality and independence of the research.

Ecological fallacy

Data are collected and analyzed at the population or group level in some investigations. For instance, researchers may examine health results, risk factor exposure, or other aspects for entire populations or geographical areas. When researchers base their conclusions or judgements about individuals within such groups only on the observed group-level data, they are committing an ecological fallacy. Problems can arise as individual-level traits or connections might be different from those observed in groups.

Regression dilution bias

When the values of the independent variable (exposure) are measured incorrectly, measurement error arises. This inaccuracy may be the result of inaccurate data acquisition techniques, tools, or participant self-reporting. When performing regression analysis, measurement error in the exposure variable tends to weaken the relationship between the exposure and the result. Thus, the expected impact of exposure on the result is less significant than the actual impact. Regression dilution bias can cause researchers to draw wrong conclusions about how strong and important a relationship between two variables is. They might overestimate the genuine effect of an exposure on the result, which affect policy, clinical practice, or public health.

SPECIAL BIAS

The Hawthorne effect

The Hawthorne effect is a psychological phenomenon that can significantly influence research outcomes. This effect occurs when participants in a study modify their behavior or performance simply because they are aware of being observed or receiving special attention. In an RCT evaluating a novel manual toothbrush design for orthodontic patients, the methodology necessitated that patients brush in front of the investigator[15]. This could have led to the Hawthorne effect as the performance of patients in home brushing may not be same as that in brushing in front of the dentist.

The Hawthorne effect can significantly influence research outcomes in various fields. For instance: (1) Bias in participant responses: Participants may alter their responses so that these are aligned with what they believe the researchers desire rather than providing genuine or accurate information; (2) Enhanced performance: Participants might perform better than they typically would because of the extra attention received during the study, leading to inflated results; (3) Social desirability bias: Participants may alter their behavior to appear more socially acceptable or desirable, resulting in responses that do not accurately reflect their usual behavior; and (4) Temporary changes: The effect is often short-lived, and once the observation or special treatment ends, participants may revert to their typical behavior.

Of these, the naturalistic observation strategy might be useful in mitigating the Hawthorne effect. It means that researchers can conduct observations unobtrusively without the participants being aware of it, thereby enhancing the authenticity of the results.

Will Rogers phenomenon

In clinical research and epidemiology, a shift in the staging or classification of people when a new diagnostic test or set of criteria is introduced is referred to as the "Will Rogers phenomenon", after the American comedian Will Rogers. This modification may appear to improve the outcomes or survival rates for some populations, but there may be no real

change in the severity or prognosis of the disease. In essence, this phenomenon illustrates the notion that "moving people across groups can improve the image of both groups". An interesting study in this regard was published by Oshman *et al* [16] in which similar patients were presented at two different timepoints to a group of specialists. When the patients' age was added at one timepoint, the agreement between the diagnosis changed significantly.

Neyman bias

Neyman bias, often referred to as "incidence–prevalence bias" or "Neyman's bias", is a statistical phenomenon that can affect how epidemiological or clinical research findings are interpreted, especially in cross-sectional and observational studies. This bias, which bears Jerzy Neyman's name, occurs when the incidence rate of a condition or disease is incorrectly estimated based on its prevalence in the studied population.

This bias develops when researchers estimate the incidence rate of a disorder based on its prevalence, particularly when the length of the condition differs among people. This approach can lead to problems because prevalence is affected by the frequency of new cases as well as the length of the illness in those patients in whom the illness is already present.

Individuals with a prolonged disease duration are more likely to be counted in the prevalent cases, which results in an overestimation of the incidence rate. In contrast, individuals with a shorter disease duration are less likely to be included, which causes the incidence rate to be underestimated.

Berkson's bias

A statistical phenomenon known as Berkson's bias, often referred to as Berkson's paradox or Berkson's fallacy, can affect how clinical research findings are interpreted, particularly in studies conducted in hospitals or using data from hospitals. When the study subjects are selected from a hospital or clinical context, Berkson's bias occurs. Owing to the fact that they are seeking medical attention or treatment, participants in these studies often have a higher risk of suffering from one or more health issues. Therefore, the correlation between risk factors and diseases may be inflated. In other words, simply because people who have the condition are more likely to be in a hospital or clinical setting, certain risk variables may seem to be more strongly connected with the disease. In a retrospective study by Jain[17], the author concluded that mandibular molars were the most frequently treated posterior teeth in both sexes and that women constituted the majority of those receiving posterior root canal therapy. However, the data were obtained from patients who reported to their college, and hence, the conclusion may not be applicable to the general population. In the discussion section, the authors compared their findings with a previous study that also had Berkson's bias. The study by Al-Negrish[18] aimed to determine the incidence and distribution of root canal treatment in a dentition of Jordanian population, but the study sample was from the department of dentistry of a hospital in Jordan.

Memory effect

Also known as recall bias or retrospective bias, it is a prevalent form of bias in research studies that can significantly impact the validity and reliability of the findings. This bias occurs when participants' memories of past events are flawed or selectively recalled, leading to inaccurate or distorted data.

In research, the memory effect can arise in various scenarios: (1) Retrospective studies: Research designs that rely on participants' recollection of past experiences, habits, or exposures are particularly vulnerable to the memory effect. Participants may inadvertently misremember or exaggerate certain events or behaviors, which can skew the results; (2) Surveys and questionnaires: When individuals are asked to report past events *via* surveys or questionnaires, their ability to accurately recall specific details can be influenced by various factors. These factors include the emotional impact of an event, the passage of time, or even external influences, such as media coverage or personal beliefs; (3) Longitudinal studies: The memory effect can occur even in longitudinal studies that track participants over an extended period. Participants might remember events differently or become influenced by subsequent experiences, leading to alterations in their responses; (4) Case–control studies: In case–control studies in which researchers compare individuals with a specific condition to those without it, recall bias can be especially problematic. Patients may be more likely to recall specific exposures or events owing to their current condition, leading to an overestimation of the association between the condition and the exposure; and (5) Self-reported data: Any study relying heavily on self-reported data is susceptible to the memory effect. People may unintentionally misrepresent their experiences or provide socially desirable responses, leading to biased conclusions.

NOVELTY BIAS

Novelty-related bias refers to the tendency of individuals, organizations, and societies to place greater emphasis and value on novel information, ideas, products, or experiences than on established or conventional ones. This bias can manifest in various aspects of human behaviors, decision-making processes, and cultural preferences. While embracing novelty can pave the way for progress and positive change, an excessive bias toward it has certain drawbacks. In academia and scientific research, there can be a pressure to publish novel findings, which might lead to a focus on flashy but potentially less rigorous or significant studies. As for the actual clinical research studies, evaluation of a novel item (*e.g.*, a fancy toothbrush design or a noncommercially available mouthwash) can also introduce a bias in that the participants use the novel product more seriously and without skipping the recommended dose or duration or both. Over the weeks, as the novelty effect fades off and opinions of clinicians might. In a recent study, comparing the manual toothbrush with a novel sonic toothbrush, the authors unknowingly introduced a potential novelty bias[7]. Had the investigators asked the patients to use the sonic toothbrush for a few weeks before starting the trial, the results might

have been different. On the contrary, some individuals may develop a bias against novelty, preferring familiar and established practices owing to a fear of the unknown or a desire for stability. This resistance to change can hinder progress and stifle innovation.

CONFIRMATION BIAS

Confirmation bias has been included under special bias category as it exerts its effect at every stage of clinical research right from study design to conducting the trial and publishing the results and even in clinical case scenarios. Researchers might design studies in a way that unconsciously favors the hypothesis they believe to be true, leading to biased data collection or experimental design. During data collection and analysis, researchers might unconsciously focus on aspects of the data that support their hypothesis while overlooking or downplaying those that are against it. After the completion of the research, there may be a tendency to submit and publish studies with positive or confirmatory results, whereas those with negative or contradictory findings might remain unpublished or receive less attention. Furthermore, confirmation bias can affect the peer review process because reviewers might be more critical of studies that challenge established beliefs and more lenient toward those that confirm prevailing notions. Clinicians involved in research or medical practice may be influenced by confirmation bias when interpreting patient data or deciding on treatments. They might give more weightage to data that support their initial diagnosis or treatment plan.

BIAS IN *IN VITRO* STUDIES

In vitro studies are experiments conducted in a controlled laboratory environment using isolated cells, tissues, or organs outside of their natural environment. While these studies are valuable for investigating specific biological mechanisms and testing hypotheses, they are not free from potential biases. Some possible biases in *in vitro* studies include the following: (1) Publication bias: *In vitro* studies that yield positive or significant results are more likely to be published than those with negative or inconclusive findings. This bias can lead to an overrepresentation of positive outcomes in the literature; (2) Selection bias: The choice of cell lines or experimental models may not entirely represent the complexity of human biology. Researchers might select cells that are easier to work with or those that support their hypothesis, potentially overlooking relevant but less convenient models; (3) Sampling bias: In *in vitro* studies, researchers often use specific cell lines or samples obtained from a specific source, such as a commercial supplier. These samples may not accurately represent the target population or disease being studied; (4) Measurement bias: Errors such as inaccurate calibration of instruments or subjective interpretation of results can occur during the measurement process. These errors can introduce discrepancies between the observed and actual outcomes; (5) Contamination or cross-contamination: *In vitro* experiments are susceptible to contamination by bacteria, fungi, or other cell lines. If not appropriately controlled, such contamination can affect the validity of the study; (6) Experimental design bias: The design of the *in vitro* study may introduce a bias. One such example is using higher concentrations of a substance to achieve more significant effects even if these concentrations are not physiologically relevant; (7) Time bias: *In vitro* studies may be conducted over a relatively short period, whereas biological processes often occur over longer timeframes. This discrepancy can lead to an incomplete understanding of the dynamics involved; and (8) Lack of ecological validity: Findings from *in vitro* studies might not translate accurately to the complexities of the human body and its interactions in the natural environment.

Hence, *in vitro* study results must be interpreted cautiously, and their limitations should be considered. To gain a more comprehensive understanding, results from such studies should be complemented with data from other research approaches, such as animal studies or clinical trials.

CONCLUSION

Researchers should be aware of these biases and adopt measures to minimize their impact to ensure the validity and reliability of their findings. Transparent reporting of study methods and potential sources of bias is crucial for the dental research community to assess the quality of the research.

FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Assessing the readability of online information about jones fracture

Khaled Farid Khaled Al-Kharouf, Faisal Idrees Khan, Greg AJ Robertson

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Khaled Farid Khaled Al-Kharouf, Orthopaedic Surgery, Portsmouth Hospitals University, Portsmouth PO6 3 LY, United Kingdom

Faisal Idrees Khan, Internal Medicine, Tunbridge Wells Hospital, Tunbridge Wells E10 5NJ, United Kingdom

Greg AJ Robertson, Orthopaedic Surgery, Queen Alexandra Hospital, Portsmouth PO6 3LY, United Kingdom

Corresponding author: Khaled Farid Khaled Al-Kharouf, MD, Doctor, Orthopaedic Surgery, Portsmouth Hospitals University, Cosham, Portsmouth PO6 3 LY, United Kingdom. kfk990@gmail.com

Abstract

BACKGROUND

Hand in hand with technological advancements, treatment modalities continue to grow. With the turn of the century, the internet has become the number one source of information for almost every topic. Thus, many patients look toward the internet as their primary source of information to learn about their respective medical conditions. The American Medical Association and National Institute of Health strongly recommend that online medical information be written at the 6th to 8th-grade level to aid comprehension by patients of all literacy backgrounds.

AIM

To assess the readability of online information regarding Jones fracture. Our hypothesis is that the reading level of medical information published on websites far exceeds the recommended reading level of 6th-8th grade as proposed by the American Medical Associate and National Institute of Health. The result of this study can help us formulate improved recommendations for publishing more comprehensible material and, thus, eventually improve patient compliance and clinical outcomes.

METHODS

The exact phrase "Jones fracture" was queried on the three most common search engines, Google, Yahoo!, and Bing, on December 28, 2022. As of December 2022, Google held 84%, Bing held 9%, and Yahoo! held 2% of the worldwide search engine market share. Web pages uniform resource locator from the first three pages of search results were recorded from each search engine. These web pages were classified according to academic, physician-sponsored, governmental and non-government organizations (NGO), commercial, and unspecified as per

formally defined categories. Websites associated with an educational institution or medical organization were classified as academic. Websites with products for sale, corporate sponsorship, or advertisements were classified as commercial. Governmental websites or NGOs comprised those that received government subsidies or grants. Webpages that were independently owned by physicians or physician groups were respectively classed as physician sponsored. The remainder of websites that did not fall under the above categories were classified as unspecified.

RESULTS

A total of 93 websites were analyzed for reading assessment. A whopping 44% of websites were commercial, followed by 22% of physician-sponsored websites. Third place belonged to non-government organization websites holding a 15% share. The academic website held a meager 9% portion, while unspecified sites were 3%. The table illustrates mean readability scores, along with average cumulative grade level. The average grade level was 10.95 ± 2.28 for all websites, with a range of 6.18 to 18.90. Since *P* values were more than 0.05, there was not a significant statistical difference between the first page results and the results of all pages. Thus, we can rationalize that readability scores are consistent throughout all pages of a website.

CONCLUSION

Hand in hand with technological advancements, treatment modalities continue to grow. With the turn of the century, the internet has become the number one source of information for almost every topic. Thus, many patients look towards the internet as the primary source of information to learn about their respective medical conditions. Our study demonstrates that current online medical information regarding Jones fracture is written at an extraordinarily high-grade level, with an average grade level of all websites at 10.95, nearly an 10th-grade educational level. The American Medical Association and National Institute of Health strongly recommend that online medical information should be written at the 6th to 8th-grade level to aid comprehension by patients of all literacy backgrounds. On the contrary, most of the medical information evaluated was at an 10th-grade level, which far exceeds recommendations by AMA and NIH. This is particularly relevant because readability scores are directly proportional to the level of comprehension attained by readers, thus directly impacting patient outcomes. In conclusion, we suggest and encourage that all online reading materials should be re-written at the 6th to 8th-grade level in a public service effort to increase compliance with treatment goals and raise awareness of preventive measures.

Key Words: Jones fracture; Jones fracture treatment; Jones fracture management; Jones fracture prevention; Jones fracture types; Jones fracture location

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Core Tip: With technological advancements, many patients look toward the internet as their primary source of information to learn about their respective medical conditions. The American Medical Association and National Institute of Health strongly recommend that online medical information be written at the 6th to 8th-grade level to aid comprehension by patients of all literacy backgrounds. Readability measures how easy a piece of text is to read. This, in turn, affects how much information people can understand and retain. Our study aims to assess the readability of online information regarding Jones fracture. A total of 93 websites were analyzed for reading assessment. The overall mean average grade level of all websites in the study was 10.95230 ± 2.27862, corresponding to a 10th-grade reading level. In Conclusion, most of the medical information evaluated was at an 11th-grade level, far exceeding AMA and NIH recommendations.

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INTRODUCTION

Sir Robert Jones originally described the Jones fracture in 1902 as a self-named fracture occurring within 3/4th of an inch from the base of the 5th metatarsal. Generally, the Jones fracture is defined as all fractures of the proximal 5th metatarsal distal to the tuberosity within 1.5 cm of this area[1]. Interestingly, the most common fracture of the foot is the Jones fracture, *i.e.*, fracture of the 5th metatarsal. Many classification systems exist describing the Jones fracture; however, the most widely used is the anatomical classification, dividing the proximal part of the 5th metatarsal into three zones[2].

Table 1 Unpaired *t*-test for overall pages *vs* first page of websites

Readability metric	Overall mean ± SD (range)	First page mean ± SD (range)	Percentage mean difference	*P* value
Flesch reading ease	55.3970 ± 12.7618 (23.2-86.1)	55.3290 ± 15.9812 (9.9-87.4)	2.32	0.553
Flesch-kincaid	10.1450 ± 2.7082 (5.0-20.1)	10.3840 ± 3.2056 (4.9-18.9)	0.47	0.915
Gunning fog	12.7830 ± 2.8099 (8.2-23.5)	12.4210 ± 3.1772 (7.6-20.7)	3.86	0.287
SMOG	9.3530 ± 1.9114 (5.6-15.8)	9.3000 ± 2.3224 (5.1-16.3)	2.27	0.523
Coleman-liau index	12.2460 ± 1.4778 (7.5-16.1)	11.9970 ± 2.2096 (8-19)	4.64	0.41
Automated readability	10.2340 ± 3.0205 (4.6-22.0)	10.5680 ± 3.6182 (3.8-19.2)	1.05	0.824
Average grade level	10.95230 ± 2.27862 (6.18-18.88)	10.93420 ± 2.73413 (6.22-18.48)	0.09	0.888

Zone 1 is the most proximal area, comprising the 5th metatarsal tuberosity and the metatarsocuboid joint. Injuries in Zone 1 are usually avulsion fractures, which are non-operatively managed to result in adequate clinical outcomes. Zone 2 consists of an area bordering the 4th and 5th intermetatarsal junction, stretching to the metaphyseal-diaphyseal junction and distal to Zone 1. A fracture in Zone 2 is classified as an actual Jones fracture. Finally, we have Zone 3, which contains the proximal 1.5 cm of the metatarsal diaphysis. Fractures in Zone 2 and Zone 3 are known to have high non-union rates with non-operative management, hence, promoting operative management as the preferred treatment[2].

With the turn of the century, the internet has become a valuable source of medical information for patients[3]. As per the 2022 Health Information National Trends Survey, approximately 74.7% of people initially browsed the internet to gain insight into their medical problems[4]. With increased online dissemination of medical information, the public has turned to the internet as their first source of information regarding medical illnesses. In fact, online resources have been shown to increase compliance with treatment goals and self-governed lifestyle changes[5].

Readability is the measure of how easy a piece of text is to read[6]. This, in turn, affects how much information people are able to understand and retain. Given that health literacy actively corresponds to positive patient outcomes, the readability of a text comes into play in how effectively a piece of information can be comprehended by patients[7]. The American Medical Association and the National Institute of Health recommend that patient education materials should be written between a 6th and 8th-grade level[8,9].

Our study aims to assess the readability of online information regarding Jones fracture. Our hypothesis is that the reading level of medical information published on websites far exceeds the recommended reading level of 6th-8th grade as proposed by the American Medical Associate and National Institute of Health. The result of this study can help us formulate improved recommendations for publishing more comprehensible material and, thus, eventually improve patient compliance and clinical outcomes.

MATERIALS AND METHODS

The exact phrase "Jones fracture" was queried on the three most common search engines, Google, Yahoo!, and Bing, on December 28, 2022. As of December 2022, Google held 84%, Bing held 9%, and Yahoo! held 2% of the worldwide search engine market share[10]. Web pages uniform resource locator (URLs) from the first three pages of search results were recorded from each search engine. These web pages were classified according to academic, physician-sponsored, governmental and non-government organizations (NGO), commercial, and unspecified as per formally defined categories[11]. Websites associated with an educational institution or medical organization were classified as academic. Websites with products for sale, corporate sponsorship, or advertisements were classified as commercial. Governmental websites or NGOs comprised those that received government subsidies or grants. Webpages that were independently owned by physicians or physician groups were respectively classed as physician sponsored. The remainder of websites that did not fall under the above categories were classified as unspecified[12-14] (Tables 1-3).

RESULTS

A total of 101 website results were obtained, 49 from Google, 28 from Yahoo!, and 24 from Bing. Only eight websites were excluded, making a total of 93 pages manually analyzed (49 from Google, 21 from Yahoo!, and 23 from Bing; Figures 1 and 2). Exclusions were made due to duplication results. All the text from the images was included as part of the analysis. On pages where there was information about multiple subjects, only information relevant to Jones fracture was selected for analysis.

Table 2 Percentage of websites and their grade level

Classification	*n*	Percentage	Average grade level	St. Dev.	Maximum	Minimum
Academic	8	0.0860	11.36	± 3.33	18.88	8.54
Physician sponsored	21	0.2258	10.93	± 1.71	13.42	8.74
Governmental & NGO	21	0.2258	11.51	± 2.56	15.68	6.18
Commercial	40	0.4301	10.84	± 2.06	18.02	6.46
Unspecified	3	0.0323	7.61	± 1.24	8.32	6.18

NGO: Non-government organizations.

Table 3 Unpaired *t*-test for government non-government organizations *vs* academic websites

Readability metric	Government & NGO mean ± SD (range)	Academic mean ± SD (range)	Percentage mean difference	*P* value
Flesch reading ease	49.0330 ± 17.4883 (28.0-86.1)	53.5880 ± 14.3224 (23.2-66.6)	4.438	0.276
Flesch-Kincaid	10.7860 ± 2.9066 (5.0-15.6)	10.825 ± 4.083 (7.5-20.1)	0.362	0.554
Gunning fog	13.529 ± 2.869 (8.2-18.5)	13.1500 ± 4.5056 (9.6-23.5)	2.838	0.475
SMOG	9.8860 ± 2.1763 (5.6-13.4)	9.7880 ± 2.8478 (7.2-15.8)	0.998	0.453
Coleman-liau index	13.1670 ± 2.2265 (7.5-16.1)	12.0500 ± 0.7051 (11.2-13.0)	8.857	0.086
Automated readability	10.200 ± 2.8660 (4.6-16.0)	11.0000 ± 4.7413 (7.2-22.0)	7.547	0.290
Average grade level	11.51330 ± 2.55634 (6.18-15.68)	11.36250 ± 3.33421 (8.54-18.88)	1.319	0.665
Complex words	130.81 ± 117.662 (44-459)	122.380 ± 51.578 (70-210)	6.663	0.304
Percentage of complex words	0.1746810 ± 0.0552751 (0.0538-0.2356)	0.1414500 ± 0.0334975 (0.1063-0.1860)	21.024	0.187
Average words per sentence	16.90710 ± 3.23038 (14.03-25.71)	20.32130 ± 8.64815 (14.12-40.74)	18.341	0.090
Average syllables per word	1.67520 ± 0.19457 (1.21-1.85)	1.56750 ± 0.07459 (1.49-1.68)	6.645	0.041

NGO: Non-government organizations.

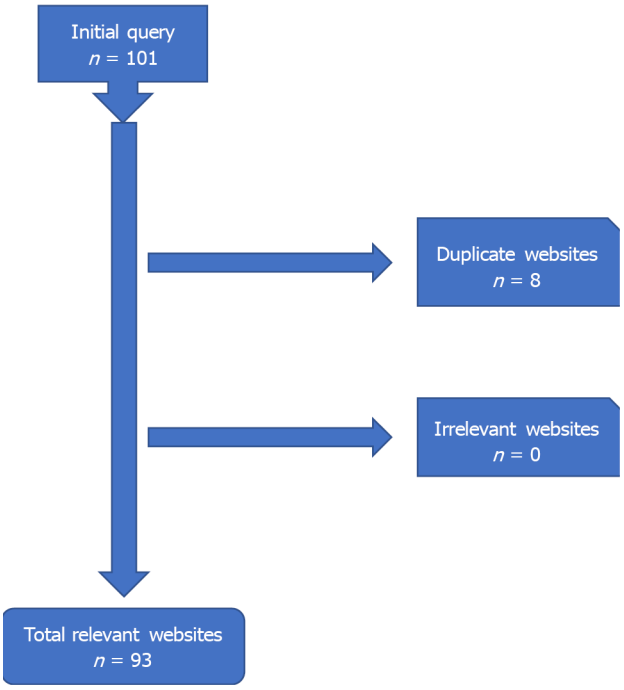
DISCUSSION

With advances in technology, operative and conservative management options for Jones continue to grow. Vast amounts of information are available on the internet. In fact, in today's age, patients tend to utilize the internet as their primary source of information before actually seeing a medical practitioner. Therefore, it is of utmost significance how medical information is disseminated to patients.

Our study aimed to assess the readability of online information regarding Jones fracture. A vast majority, 77%, of websites were written above an eighth-grade level, far surpassing recommendations set by AMA and NIH[15-17]. The overall mean average grade level of all websites in the study was 10.9523 ± 2.27862, corresponding to a 10th-grade reading level. Strikingly, governmental and NGO websites were found to have the highest average grade level of 11.51 ± 2.56, representing an 11th-grade level of comprehension. Even though previous studies[18] have shown Academic websites to have the high readability score, in our case, academic websites ranked 2nd with an average grade level of 11.36 ± 3.33, which was still an eleventh-grade reading level. Considering these results, full comprehension of online medical information would require the completion of at least a secondary education.

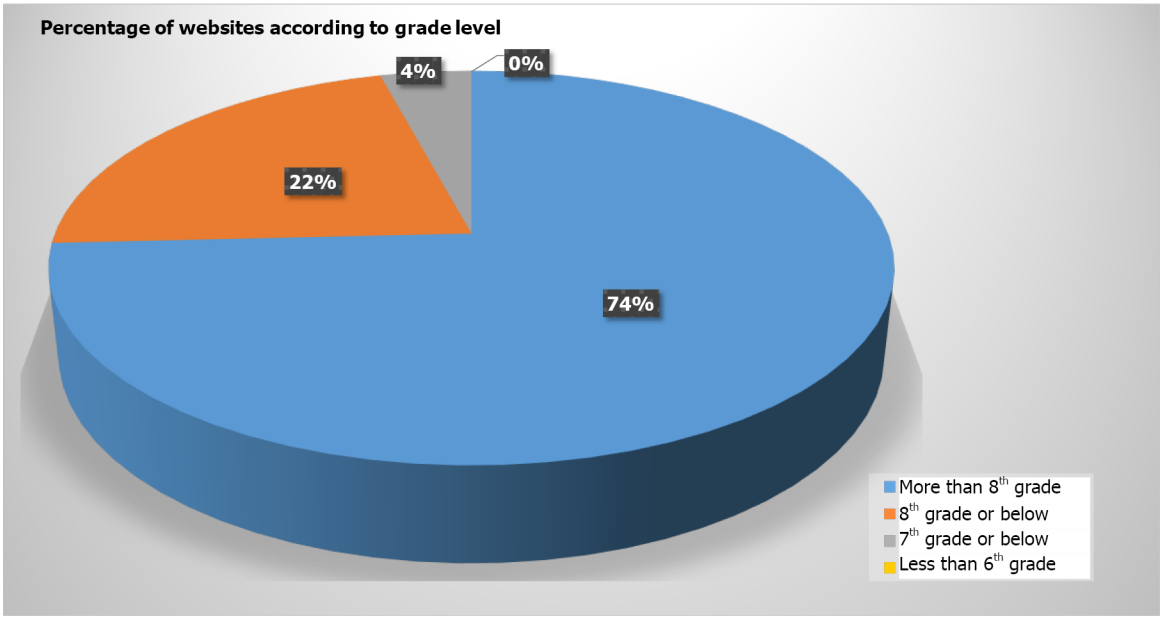
CONCLUSION

Hand in hand with technological advancements, treatment modalities continue to grow. With the turn of the century, the internet has become the number one source of information for almost every topic. Thus, many patients look towards the internet as the primary source of information to learn about their respective medical conditions. Our study demonstrates that current online medical information regarding Jones fracture is written at an ex-traordinarily high-grade level, with an average grade level of all websites at 10.95, nearly an 10th-grade educational level. The American Medical Association and National Institute of Health strongly recommend that online medical information should be written at the 6th to 8th-



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Figure 1 Flow diagram of inclusion and exclusion criteria.



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Figure 2 Percentage of websites and their grade level.

grade level to aid comprehension by patients of all literacy backgrounds. On the contrary, most of the medical information evaluated was at an 10th-grade level, which far exceeds recommendations by AMA and NIH. This is particularly relevant because readability scores are direct-ly proportional to the level of comprehension attained by readers, thus directly impacting patient outcomes. In conclusion, we suggest and encourage that all online reading materials should be re-written at the 6th to 8th-grade level in a public service effort to increase compliance with treatment goals and raise awareness of preventive measures.

ARTICLE HIGHLIGHTS

Research background

Hand in hand with technological advancements, treatment modalities continue to grow. With the turn of the century, the internet has become the number one source of information for almost every topic. Thus, many patients look toward the internet as their primary source of information to learn about their respective medical conditions. The American Medical Association and National Institute of Health strongly recommend that online medical information be written at the 6th to 8th-grade level to aid comprehension by patients of all literacy backgrounds.

Research motivation

With advances in technology, operative and conservative management options for Jones continue to grow. Vast amounts of information are available on the internet. In fact, in today's age, patients tend to utilize the internet as their primary source of information before actually seeing a medical practitioner. Therefore, it is of utmost significance how medical information is disseminated to patients.

Research objectives

To assess the readability of online information regarding Jones fracture. Our hypothesis is that the reading level of medical information published on websites far exceeds the recommended reading level of 6th-8th grade as proposed by the American Medical Associate and National Institute of Health. The result of this study can help us formulate improved recommendations for publishing more comprehensible material and, thus, eventually improve patient compliance and clinical outcomes.

Research methods

The exact phrase "Jones fracture" was queried on the three most common search engines, Google, Yahoo!, and Bing, on December 28, 2022. As of December 2022, Google held 84%, Bing held 9%, and Yahoo! held 2% of the worldwide search engine market share. Web pages uniform resource locator from the first three pages of search results were recorded from each search engine. These web pages were classified according to academic, physician-sponsored, governmental and non-government organizations (NGO), commercial, and unspecified as per formally defined categories. Websites associated with an educational institution or medical organization were classified as academic. Websites with products for sale, corporate sponsorship, or advertisements were classified as commercial. Governmental websites or NGOs comprised those that received government subsidies or grants. Webpages that were independently owned by physicians or physician groups were respectively classed as physician sponsored. The remainder of websites that did not fall under the above categories were classified as unspecified.

Research results

A total of 101 website results were obtained, 49 from Google, 28 from Yahoo!, and 24 from Bing. Only eight websites were excluded, making a total of 93 pages manually analyzed (49 from Google, 21 from Yahoo!, and 23 from Bing). Exclusions were made due to duplication results. All the text from the images was included as part of the analysis. On pages where there was information about multiple subjects, only information relevant to Jones fracture was selected for analysis.

Research conclusions

Hand in hand with technological advancements, treatment modalities continue to grow. With the turn of the century, the internet has become the number one source of information for almost every topic. Thus, many patients look towards the internet as the primary source of information to learn about their respective medical conditions. Our study demonstrates that current online medical information regarding Jones fracture is written at an extraordinarily high-grade level, with an average grade level of all websites at 10.95, nearly an 10th-grade educational level. The American Medical Association and National Institute of Health strongly recommend that online medical information should be written at the 6th to 8th-grade level to aid comprehension by patients of all literacy backgrounds. On the contrary, most of the medical information evaluated was at an 10th-grade level, which far exceeds recommendations by AMA and NIH. This is particularly relevant because readability scores are directly proportional to the level of comprehension attained by readers, thus directly impacting patient outcomes. In conclusion, we suggest and encourage that all online reading materials should be re-written at the 6th to 8th-grade level in a public service effort to increase compliance with treatment goals and raise awareness of preventive measures.

Research perspectives

We suggest and encourage that all online reading materials should be re-written at the 6th to 8th-grade level in a public service effort to increase compliance with treatment goals and raise awareness of preventive measures.

FOOTNOTES

Author contributions: Al-Kharouf KFK conceived the methodology for the manuscript, performed the literature search and analysis for the study, and wrote the manuscript; Khan FI performed the literature search and analysis for the study and wrote the manuscript; Robertson GA advised on the study, and reviewed and edited the manuscript.

Institutional review board statement: Not required, no human or animal involved in our study.

Informed consent statement: The dataset consisted of anonymized synthesize evidence from published studies. Thus, no informed consent for data sharing was required.

Conflict-of-interest statement: Khaled Farid Khaled Al-Kharouf, Faisal Idrees Khan, and Greg Robertson have no conflicts of interest to declare. None have received fees for serving as a speaker or a consultant for commercial organizations. None have received research funding from commercial organizations. All are employees of the UK National Health Service, though not of any commercial organizations. None own stocks or shares in related commercial organizations. None own patent related to the topic of this study.

Data sharing statement: Technical appendix and datasets are available from the corresponding author at kfk990@gmail.com.

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Country/Territory of origin: United Kingdom

ORCID number: Khaled Farid Khaled Al-Kharouf 0009-0001-6559-6154; Greg AJ Robertson 0000-0002-9152-7144.

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ORIGINAL ARTICLE

Retrospective Study

Impact of COVID-19 lockdown on hospital admissions for epistaxis in Germany

Adrian Hoenle, Martin Wagner, Stephan Lorenz, Helmut Steinhart

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Adrian Hoenle, Martin Wagner, Helmut Steinhart, Department of Ear, Nose and Throat, Head and Neck Surgery, Marienhospital Stuttgart, Stuttgart 70199, Germany

Stephan Lorenz, Department of Emergency Medicine, Katharinenhospital Stuttgart, Stuttgart 70174, Germany

Corresponding author: Adrian Hoenle, BSc, MD, Doctor, Surgeon, Department of Ear, Nose and Throat, Head and Neck Surgery, Marienhospital Stuttgart, Boeheimstrasse 37, 70199 Stuttgart, Germany, Stuttgart 70199, Germany. adrian.hoenle@gmx.net

Abstract

BACKGROUND

Reports of a decrease in hospital admissions during the coronavirus disease 2019 (COVID-19) lockdown period have raised concerns about delayed or missed diagnoses and treatments for non-COVID-19-related illnesses.

AIM

To investigate the impact of the COVID-19 pandemic-induced lockdown and its end on hospital admissions of patients with epistaxis in Germany.

METHODS

A retrospective analysis based on the national database of the Hospital Remuneration System was used to compare hospital admissions during defined time periods between 2019 and 2022 with the lockdown period as the reference period. This was done on a weekly basis before, during, and after the lockdown. An Interrupted Time Series was used as the analysis method.

RESULTS

In our analysis, we included 26183 patients. The implementation of the lockdown led to a substantial reduction in the overall occurrence of epistaxis among patients ($P < 0.05$). This effect was most pronounced in the age group of 0-39 years, where the decrease was highly significant ($P < 0.001$). However, there was no change observed in patients aged 80 years and older (not significant). With the end of the lockdown period, the overall number of patients, especially in the youngest age group, increased abruptly and significantly ($P < 0.01$).

CONCLUSION

During the lockdown period, there was a decrease in hospital admissions for

younger patients with epistaxis, possibly due to the fear of COVID-19 exposure. We also conclude that the severity of epistaxis was not underestimated in the elderly during the pandemic.

Key Words: COVID-19; Epistaxis; Lockdown; Pandemic; Emergency medicine; Otolaryngology

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Core Tip: The coronavirus disease 2019 (COVID-19) pandemic has had a significant impact on healthcare systems worldwide. In an effort to reduce the spread of the virus, many countries have implemented lockdown measures that restrict movement and social interaction. While these measures have been effective in reducing the transmission of COVID-19, they have also had unintended consequences on healthcare delivery and hospital admissions. Several studies have reported a decrease in hospital admissions during the COVID-19 lockdown period. We showed that the pandemic-induced lockdown resulted in a direct decrease in hospitalizations especially for young patients with epistaxis and an immediate increase in hospitalizations with its end. This might be caused by fear of exposure to COVID-19, unintended consequences of public health recommendations to minimize non-urgent healthcare, or stay at home orders. These findings match with results from previous studies. Conversely, these measures did not lead to any change in older patients, which suggests that at least in this age group, the symptoms of epistaxis should not be underestimated, even with regard to a possible exposure to the coronavirus.

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INTRODUCTION

Epistaxis

Epistaxis is a common emergency in the field of ear, nose, and throat, with varying degrees of severity. Roughly 60% of the population are expected to encounter it at least once during their lifetime[1]. The causes of epistaxis can range from idiopathic to cancerous lesions, with only about 6% of cases requiring medical or surgical attention[2,3]. Idiopathic or spontaneous factors are the primary cause of epistaxis, constituting the cause in at least 70% of cases. These occurrences are frequently associated with conditions such as hypertension, atherosclerotic disease, smoking, or the use of oral anticoagulation medications[4]. Age has also been shown to be a factor in the incidence of epistaxis, with the risk increasing as individuals get older[5].

The Little area, located along the anterior septum, is the origin of approximately 90% of epistaxis cases[6]. Blood supply to this region is provided by the Kiesselbach plexus, composed of second-order branches from both the external and internal carotid arteries[7]. Hemorrhage in this area is commonly referred to as anterior epistaxis and can often be managed using conservative approaches like nostril pressure, topical vasoconstrictors or hemostatic agents, cryotherapy, electrocautery, or anterior nasal packing[8].

In contrast, posterior epistaxis, accounting for only 5% to 10% of cases, originates from the more posterior regions of the nasal cavity[9]. Managing posterior-based nasal bleeding with anterior and posterior nasal packs is less successful, with success rates ranging from 48% to 83%[10-12]. In some cases, nasal hemorrhage persists despite packing or recurs upon pack removal. Posterior epistaxis can be effectively treated through endoscopic or open surgical approaches involving direct ligation or cauterization of the affected artery, with a reported success rate of 97%[13,14].

Endovascular embolization is another viable option to halt nasal bleeding, with reported success rates ranging from 71% to 100%. However, this approach may entail minor complications, such as septal perforation, sinusitis, headache, facial or jaw pain, and facial edema[2,13]. More serious complications, including stroke, facial nerve paresis, soft-tissue necrosis, and even blindness, can occur as a result of inadvertent embolization[14-16].

Recent research indicates that epistaxis may serve as an initial symptom of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. This virus can affect the nasal epithelium, potentially increasing the risk of developing epistaxis[17,18].

Hospital admissions during coronavirus disease 2019 pandemic

The global healthcare systems have been profoundly affected by the coronavirus disease 2019 (COVID-19) pandemic. To mitigate the virus's spread, numerous nations have enacted lockdown measures, curtailing mobility and social interactions. While these steps have effectively curbed COVID-19 transmission, they have also led to unintended repercussions for healthcare provision and hospital admissions. Several studies have reported a decrease in hospital admissions during the COVID-19 lockdown period[19,20]. This decrease has been attributed to several factors, including the cancellation of elective surgeries, reduced emergency department visits, and a decrease in the incidence of some

illnesses due to lifestyle changes[21-23].

While the decrease in hospital admissions during the COVID-19 lockdown period may seem like a positive outcome, it has raised concerns about delayed or missed diagnoses and treatments for non-COVID-19-related illnesses. This could potentially result in long-term health consequences for patients[24].

Aim of the study

The aim of this study was to examine whether there was a decline in hospital admissions for epistaxis in Germany during the COVID-19 lockdown. Given the potential impact of the COVID-19 pandemic on healthcare delivery and the need for timely treatment of epistaxis to prevent complications, it is important to investigate whether there has been a decline in hospital admissions for this condition during the lockdown period. By examining hospital admission rates for epistaxis during the COVID-19 lockdown period and comparing them to pre-lockdown rates, we can determine whether there was a significant decrease in hospital admissions for this condition.

Motivated by the pressing need to understand how the COVID-19 pandemic might have influenced the healthcare-seeking behavior of individuals, the authors of this study meticulously collected and analyzed nationwide data. The authors recognized that fear of potential coronavirus exposure could have deterred patients from seeking necessary medical attention, even for severe conditions such as epistaxis. This study not only highlights the authors' commitment to addressing a critical gap in medical research during a global crisis but also showcases their dedication to ensuring comprehensive and accurate data collection.

This approach is positioned in the temporal course prior to the outcome following the inpatient admission and can therefore be considered as a complement to other recently published studies that have dealt with patient outcomes after coronavirus infection. In this domain, models using machine learning approaches have been introduced to, for example, estimate the mortality risk of patients with pre-existing diabetes based on various input parameters or to perform early classification of COVID-19 patients through deep learning techniques[25,26].

The findings of this study will provide valuable insights into the impact of the COVID-19 pandemic on the management of epistaxis and may inform the development of strategies to ensure timely access to care for patients with this condition. Furthermore, the authors' contributions extend beyond the immediate scope of the research by shedding light on the broader challenges of maintaining regular healthcare services during times of crisis. Through this study, the authors aim to support healthcare systems in adapting to unforeseen disruptions and guaranteeing that patients receive the essential care that they require, irrespective of external circumstances.

MATERIALS AND METHODS

Patient population

This epidemiological retrospective observational study was performed by using quasi-anonymous open-access population data from the Institute for the Hospital Remuneration System in Germany[27]. This database was used to access the weekly number of hospital admissions for patients with epistaxis (ICD R04.0), with the ICD-10 diagnosis not differentiating between the cause and type of epistaxis (for example anterior or posterior). All patients of all ages and gender who were admitted within Germany with the diagnosis of epistaxis during the specified period were included.

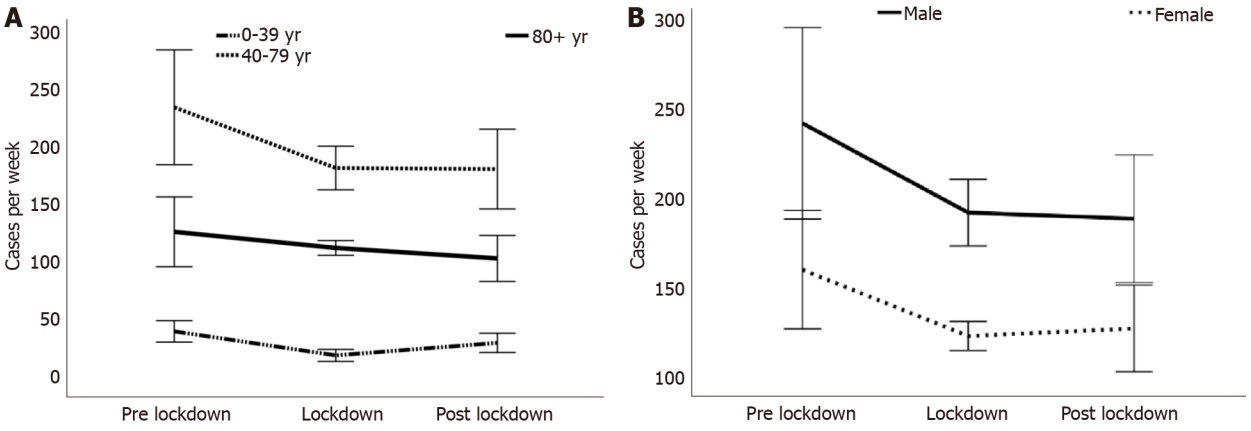
The study period extended from February 1st to June 8th of the years 2019 to 2022. This period was divided into 6-wk blocks, with the government-initiated coronavirus lockdown from March 15, 2020 to April 26, 2020 forming a separate group (lockdown). This resulted in a uniform study period of 18 wk per year to rule out the seasonal incidence of epistaxis[28-30]. Cases before March 15, 2020 (2020 wk 12) were classified into the pre-lockdown group, and cases after April 26, 2020 (2020 wk 18) were classified into the post-lockdown group. These respective time periods of inpatient admission were compared to investigate whether there have been time-dependent changes in patient numbers as the outcome of interest.

Statistical analysis

The average number of hospital admissions for epistaxis per week was calculated according to time period, gender, and age. Depending on age, patients were grouped into 0-39 years, 40-79 years, or 80 years and older. To evaluate the data, weekly case numbers were presented using an interrupted time series (ITS) analysis. This is a quasi-experimental design suitable for measuring the population-level impact of healthcare interventions[31,32]. The ITS was presented in tabular form. An AutoRegressive Integrated Moving Average (ARIMA) forecast model without seasonal effects was used as a counterfactual in order to provide a more accurate estimate of what would have happened in the absence of the intervention than linear regression[33]. The counterfactual was calculated from the pre-lockdown group as well as from the lockdown group. The results are presented as a percentage deviation from the predicted value, with the respective time boundaries of the Interrupted Time Series corresponding to the start and end of the COVID-19 lockdown.

Values are reported as absolute numbers (*n*), mean, standard deviation (SD), and 95% confidence interval (95%CI). Due to the metric scaling, analysis of variance was performed to test for mean differences. A *t*-test for independent samples was used as a *post-hoc* test with Bonferroni correction to avoid alpha error accumulation[34].

A P value of $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS, version 29.0.0.0 (IBM Corporation, Armonk, NY, United States) and Microsoft Excel, version 2019 (Microsoft Corporation, Redmond, WA, United States).



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Figure 1 Cases per week grouped by time period presented as the mean and standard deviation. A: Age-dependent; B: Male and female.

The recommendations for good practice in secondary data analysis established by the German Working Group on the Collection and Use of Secondary Data were taken into full account. According to the Professional Code of Conduct of the Regional Medical Association, the study did not require ethical approval since it did not involve the use of identifiable patient data.

RESULTS

Overall, 26183 inpatient cases were included in the analysis with a male-to-female ratio of 1.51. The largest age group was the 40-79-year-old group (n = 15145; 58%), followed by those over 80 years of age (n = 8526; 33%). The smallest group was made up of patients aged up to 39 years old (n = 2512; 9%).

It was found that regardless of age and gender, most weekly admissions occurred during the 2019 study period. In contrast, 2020 marked the lowest weekly case numbers in the group up to 39 years compared to the other study periods (P < 0.001). In the age group of 40-79 years, the highest case numbers were also observed in 2019 (P < 0.001), with a statistically constant value in the following years. Only in the group of those over 80 years old, a statistically constant weekly case number was observed for each study year.

Fragmented into 12 equally sized time periods (Table 1), the weekly caseloads for epistaxis were significantly different compared to the restriction period (P < 0.001).

Cases had decreased by 22% from 401.5 (SD 84.2) in the pre-lockdown period to 314.2 (SD 20.6) during the lockdown period, irrespective of age and gender (P < 0.05). Subsequently, there was a marginal, non-significant increase of 0.3% to 315.0 (SD 56.2). Notably, Figure 1 illustrates that the majority of patients were middle-aged and male.

When considering gender, a notable decrease of 29% (P < 0.01) was observed during the lockdown period for female patients, while male patients experienced a decrease of 21%. In the post-lockdown period, cases increased by 11% among females, whereas male patients showed a slight additional decrease of 2% (Table 2, Figure 1B).

The male-to-female ratio remained approximately constant during the individual study periods. During the lockdown period, there was an increase of 3.4%, and during the subsequent post-lockdown period, there was a decrease of 4.8% (not significant; Table 2).

The lockdown period led to a significant decrease in epistaxis cases, especially among young people (0-39 years of age), with a reduction of 51% (P < 0.001). After the end of the restriction period, there was a clear and continuing statistically significant, increase in patient numbers by 50% (P < 0.01). In the age group of 40-79 years, there was also a significant decrease in the number of cases by 22% (P < 0.01) with the start of the lockdown period, which remained constant thereafter. Only in the group of the oldest patients (80+ years of age), a statistically constant weekly number of cases was observed. This number decreased by 11% at the beginning of the lockdown period and then by an additional 8% (Table 2, Figure 1A).

These observations are also consistent with the results of the ITS. Compared to the actual case numbers, the counterfactual for the lockdown resulted in a significantly higher number, which was also most pronounced in the youngest age group. With the end of the lockdown period, as described above, there was again an increase in patient numbers. Based on the estimated value from the counterfactual calculated from the lockdown period, there were also significant deviations from the actual case numbers during the post-lockdown period. Here too, the effect was strongest in the youngest age group (Table 3, Figure 2).

DISCUSSION

This study aimed to investigate the influence of the nationwide Corona lockdown on hospital admissions pertaining to

Table 1 Total cases of patients with epistaxis in Germany

Category		All	Gender		Age group (yr)		
			Female	Male	0-39	40-79	80+
February 1, 2019- March 14, 2019	*N*	2620	1075	1545	249	1580	791
	Cases per week		179.3 (36.8)	257.5 (70.0)	41.5 (8.2)	263.3 (62.9)	131.8 (37.8)
March 15, 2019-April 26, 2019	*N*	3082	1201	1881	301	1768	1013
	Cases per week		171.4 (16.3)	268.7 (20.6)	43.0 (8.6)	252.6 (17.6)	144.7 (8.1)
April 27, 2019-June 8, 2019	*N*	2210	920	1290	233	1297	680
	Cases per week		153.3 (14.6)	215.0 (24.5)	38.8 (8.7)	216.2 (24.4)	113.3 (13.9)
February 1, 2020-March 14, 2020	*N*	2526	955	1571	269	1449	808
	Cases per week		136.4 (42.3)	224.4 (69.7)	38.4 (12.4)	207.0 (61.2)	115.4 (40.6)
March 15, 2020-April 26, 2020 (lockdown)	*N*	1885	735	1150	118	1091	676
	Cases per week		122.5 (8.1)	191.7 (18.7)	19.7 (5.2)	181.8 (18.8)	112.7 (6.4)
April 27, 2020-June 8, 2020	*N*	1790	718	1072	123	2090	587
	Cases per week		119.7 (20.8)	178.7 (15.4)	20.5 (6.0)	180.0 (19.7)	97.8 (12.2)
February 1, 2021-March 14, 2021	*N*	2042	853	1189	166	1185	691
	Cases per week		142.2 (9.2)	198.2 (24.2)	27.7 (6.4)	197.5 (13.9)	115.2 (8.5)
March 15, 2021-April 26, 2021	*N*	2127	889	1238	218	1226	683
	Cases per week		148.2 (12.2)	206.3 (7.6)	36.3 (8.6)	204.3 (11.6)	113.8 (14.2)
April 27, 2021-June 8, 2021	*N*	1998	808	1190	217	1086	695
	Cases per week		115.4 (32.5)	170.0 (54.3)	31.0 (8.6)	155.1 (48.8)	99.3 (30.4)
February 1, 2022-March 14, 2022	*N*	2046	812	1234	184	1193	669
	Cases per week		135.3 (19.5)	205.7 (35.0)	30.7 (5.7)	198.8 (34.4)	111.5 (15.5)
March 15, 2022-April 26, 2022	*N*	1953	753	1200	213	1096	644
	Cases per week		125.5 (11.7)	200.0 (22.3)	35.5 (7.5)	182.7 (15.3)	107.3 (6.0)
April 27, 2022-June 8, 2022	*N*	1904	743	1161	221	1094	589
	Cases per week		106.2 (27.0)	165.9 (46.3)	31.6 (10.1)	156.3 (42.0)	84.1 (22.9)

Cases per week: Mean, standard deviation (SD).

epistaxis. Therefore, in addition to the 6-wk lockdown, hospital admissions from 2019 to 2022 were also investigated nationwide. This was done over a total period of 18 wk per year to compensate for seasonal differences in the incidence of epistaxis. From our point of view, epistaxis is a clear and serious symptomatology, while other diseases with ambiguous symptoms such as stroke could be underestimated in terms of possible exposure to the coronavirus[35-38]. The decrease in patients with various diagnoses during the Corona lockdown has already been reported in numerous studies[39-44].

The majority of patients in this study were between 40 and 79 years old and male. This is consistent with previous publications that have associated older age and male gender with a higher incidence of epistaxis[4,45-47].

It was shown that the introduction of the COVID-19 lockdown was associated with a decrease in nationwide hospital admissions for epistaxis. This was not dependent on gender. However, a strongly significant decrease was observed in the youngest age group of 0-39 years. In the oldest age group of 79 years and older, there was no significant decrease in the number of patients. With the end of the COVID-19 lockdown 6 wk later, the numbers significantly increased again in the age group up to 39 years, while no significant changes were observed in patients aged 40 years and older.

Using ITS analysis, a clear association could be demonstrated both with the start and the end of the COVID-19 lockdown. The difference to the counterfactual was most pronounced in the youngest patient group, in line with the significance described above. It can be inferred from this that both the start and end of the COVID-19 lockdown had a direct influence on the number of hospital admissions.

The significant decrease in patients with epistaxis in the youngest age group could be due, on the one hand, to a greater fear of exposure to the coronavirus in this group. Another reason could be the less severe symptomatology on average in younger patients[48-50]. This is supported by the subsequent significant increase in patient numbers in this age group after the lockdown, which was also shown in the ITS and was associated with it.

Table 2 Total cases of patients in regard to the coronavirus disease 2019 lockdown in Germany

Category	Pre-lockdown			Lockdown		Post-lockdown		
	Cases per week	95%CI	*P* value	Cases per week	95%CI	Cases per week	95%CI	*P* value
Total	401.5 (84.2)	367.5-435.5	0.05	314.2 (20.6)	292.5-335.8	315.0 (56.2)	297.9-332.1	NS
Female	159.7 (33.1)	146.3-173.0	0.01	112.5 (8.1)	113.9-131.0	126.7 (24.3)	119.3-134.1	NS
Male	241.8 (53.7)	220.1-263.5	NS	191.7 (18.7)	172.1-211.2	188.3 (35.8)	117.4-199.2	[a]
MFR	1.52 (0.18)	1.45-1.59	NS	1.57 (0.18)	1.38-1.76	1.50 (0.22)	1.43-1.56	NS
0-39	40.4 (9.3)	36.7-44.2	[a]	19.7 (5.2)	14.2-25.1	30.5 (8.3)	27.7-33.0	0.01
40-79	234.4 (49.7)	214.3-254.5	0.01	181.8 (18.8)	162.0-201.6	180.9 (34.5)	170.4-191.4	NS
80+	126.6 (30.2)	114.4-138.8	NS	112.7 (6.4)	105.9-119.4	103.6 (19.9)	97.5-109.6	NS

[a]$P < 0.001$.

Cases per week: Mean, standard deviation (SD); Reference period: Lockdown group, MFR: Male-to-female ratio; NS: Not significant.

Table 3 Interrupted time series analysis

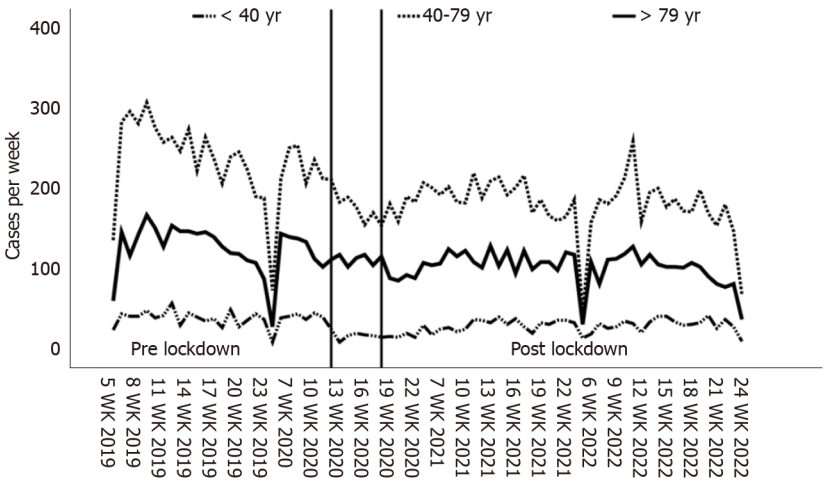
Category	Lockdown			Post-lockdown		
	Cases per week	95%CI	$\Delta_{pred-rep}$ (%)[a]	Cases per week	95%CI	$\Delta_{pred-rep}$ (%)[b]
Total						
Reported	314.2 (20.6)	292.5-335.8		315.0 (56.2)	297.9-332.1	
Predicted	364.7	152.8-576.9	+13.9	253.3	214.9-291.7	-24.5
Female						
Reported	112.5 (8.1)	113.9-131.0		126.7 (24.3)	119.3-134.1	
Predicted	126.3	67.5-185.1	+12.3	123.0	102.0-143.0	-3.0
Male						
Reported	191.7 (18.7)	172.1-211.2		188.3 (35.8)	117.4-199.2	
Predicted	242.0	131.0-352.0	+26.3	139.1	100.0-178.1	-35.4
0-39						
Reported	19.7 (5.2)	14.2-25.1		30.5 (8.3)	27.7-33.0	
Predicted	41.3	23.9-59.3	+52.3	20.0	6.0-33.0	-52.5
40-79						
Reported	181.8 (18.8)	162.0-201.6		180.9 (34.5)	170.4-191.4	
Predicted	212.1	92.3-332.5	+14.3	127.4	89.4-165.1	-42.0
80+						
Reported	112.7 (6.4)	105.9-119.4		103.6 (19.9)	97.5-109.6	
Predicted	127.0	64.0-189.0	+11.3	113.0	86.0-129.0	+8.3

[a]Based on the counterfactual calculated from the pre-lockdown period.

[b]Based on the counterfactual calculated from the lockdown period. Cases per week: Mean, standard deviation (SD).

Limitations

First, the hospital data analyzed represent a comprehensive inquiry into inpatient admissions for epistaxis in Germany. However, no data on outpatient visits to emergency departments are available in the dataset. Second, adjustments for comorbidities, socio-economic factors, or place of residence were not feasible due to the absence of these patient-level data. Third, while coding issues might have arisen for different diagnoses, we determined that these were unlikely to be a significant confounding factor, given the size of the population.



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Figure 2 Hospital admissions by age. Vertical lines indicate the beginning and end of the coronavirus disease 2019 lockdown.

CONCLUSION

Our study demonstrated that, based on actual case numbers and simulated calculations using ARIMA forecast along with ITS analysis, the pandemic-induced lockdown led to a direct reduction in hospitalizations, particularly among young patients with epistaxis, and an immediate surge in hospitalizations upon its termination. This could be attributed to concerns about COVID-19 exposure, unanticipated outcomes of public health advice to reduce non-urgent healthcare visits, or adherence to stay-at-home orders, aligning with findings from prior research[39-44].

Conversely, these measures did not lead to any change in older patients, which implies that at least in this age group, the symptoms of epistaxis should not be underestimated, even with regard to a possible exposure to the coronavirus.

These findings highlight how a nationwide intervention, such as the COVID-19 lockdown in this instance, can directly influence hospital admissions within specific segments of the population.

ARTICLE HIGHLIGHTS

Research background

The COVID-19 pandemic has had a profound impact on global healthcare systems, leading many nations to enforce lockdowns that restrict movement and social interactions in an effort to curb virus transmission. Although effective against COVID-19, these measures have inadvertently affected healthcare delivery and hospital admissions. Numerous studies have noted a decline in hospital admissions during the lockdown, attributed to factors such as postponed elective surgeries, decreased Emergency Room visits, and lifestyle-related illness reductions. Despite the apparent benefits of reduced admissions, apprehensions arise over potential long-term health implications due to delayed diagnoses and treatments for non-COVID-19 conditions.

Research motivation

Concerns have arisen due to reports of reduced hospital admissions during the COVID-19 lockdown, suggesting potential delays or omissions in diagnosing and treating non-COVID-19-related illnesses. The decrease in hospital visits during this period has sparked worries about the impact on timely medical interventions. The lockdown's effect on hospital admissions has prompted discussions regarding possible disruptions to the identification and management of non-COVID-19-related medical conditions.

Research objectives

To examine how the COVID-19 pandemic-induced lockdown and its conclusion affected hospital admissions among patients with epistaxis in Germany.

Research methods

Utilizing quasi-anonymous open-access data from Germany's Institute for the Hospital Remuneration System, this retrospective observational study analyzed hospital admissions for epistaxis, considering patient age and gender. The study covered February 1 to June 8 from 2019 to 2022, segmented into six-week periods, with a distinct lockdown group from March 15 to April 26, 2020. Statistical analysis employed Interrupted Time Series and AutoRegressive Integrated Moving Average models, presenting deviations from predicted values. Ethical approval was not necessary due to the absence of identifiable patient data, aligned with ethical guidelines.

Research results

In total, 26183 inpatient cases were analyzed, with a male-to-female ratio of 1.51. The 40-79-year-old group (n = 15145; 58%) had the most cases, followed by those over 80 years (n = 8526; 33%), and the smallest group was aged up to 39 years (n = 2512; 9%). Weekly admissions peaked in 2019 across age and gender groups, while the 2020 lockdown period saw the lowest weekly case numbers for those under 39 years ($P < 0.001$). In the age group of 40-79 years, 2019 had the highest case numbers ($P < 0.001$), remaining constant in subsequent years; those over 80 years showed consistent weekly case numbers. Fragmented into 12 time periods, weekly epistaxis cases significantly differed during the restriction ($P < 0.001$). There was a 22% decrease in cases during the lockdown ($P < 0.05$), followed by a slight increase of 0.3%, particularly affecting middle-aged males. Lockdown caused a notable 29% decrease in female cases ($P < 0.01$) and 21% in males, with an 11% post-lockdown increase in females and 2% decrease in males. The male-to-female ratio remained stable. Lockdown led to a significant 51% decrease in young patients (0-39 years, $P < 0.001$) and a subsequent 50% increase ($P < 0.01$), while the 40-79 age group had a 22% decrease ($P < 0.01$) and the oldest group remained constant. These trends were consistent with ITS results, showcasing the impact on different age groups.

Research conclusions

Our study demonstrated that the pandemic-induced lockdown led to a direct decrease in hospitalizations, particularly among young patients with epistaxis. This was followed by a rapid increase after the lockdown was ended. Possible factors contributing to this trend include COVID-19-related fears, unintended consequences of healthcare recommendations, or stay-at-home orders – findings that align with previous research. Notably, older patients were not similarly affected, highlighting the importance of addressing epistaxis symptoms, even in the context of potential COVID-19 exposure. These results emphasize the significant impact of a nationwide intervention like the COVID-19 lockdown on hospital admissions in specific demographic groups.

Research perspectives

Further and more comprehensive research based on larger datasets is necessary to obtain insights into lockdown-induced changes in hospital admissions for other diagnoses as well.

FOOTNOTES

Author contributions: Hoenle A designed the study, collected and analyzed the data, and wrote and edited the manuscript; Wagner M and Lorenz S collected data for the manuscript; Steinhart H assisted in study design and edited the manuscript.

Institutional review board statement: The study was reviewed and approved by the Ethikkommission Marienhospital Stuttgart Institutional Review Board.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous secondary clinical data which is publicly available.

Conflict-of-interest statement: All the authors report no conflicts of interest for this article.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at adrian.hoenle@gmx.net. Participants gave informed consent for data sharing.

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Country/Territory of origin: Germany

ORCID number: Adrian Hoenle 0000-0002-6608-0782.

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ORIGINAL ARTICLE

Retrospective Study

Effect of vaccination status on CORADS and computed tomography severity score in hospitalized COVID-19 patients: A retrospective study

Umut Devrim Binay, Erdal Karavaş, Faruk Karakeçili, Orçun Barkay, Sonay Aydin, Düzgün Can Şenbil

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Umut Devrim Binay, Faruk Karakeçili, Orçun Barkay, Department of Infectious Diseases and Clinical Microbiology, Erzincan Binali Yildirim University, Faculty of Medicine, Erzincan 24000, Turkey

Erdal Karavaş, Department of Radiology, Bandırma 17 Eylül University, Faculty of Medicine, Balıkesir 10200, Turkey

Sonay Aydin, Düzgün Can Şenbil, Department of Radiology, Erzincan Binali Yıldırım University, Faculty of Medicine, Erzincan 24000, Turkey

Corresponding author: Umut Devrim Binay, MD, Assistant Professor, Department of Infectious Diseases and Clinical Microbiology, Erzincan Binali Yildirim University, Faculty of Medicine, Başbağlar Mahallesi, Hacı Ali Akın Sokağı, Erzincan 24000, Turkey.
umut.binay@erzincan.edu.tr

Abstract

BACKGROUND

The coronavirus disease 2019 (COVID-19) pandemic is continuing. The disease most commonly affects the lungs. Since the beginning of the pandemic thorax computed tomography (CT) has been an indispensable imaging method for diagnosis and follow-up. The disease is tried to be controlled with vaccines. Vaccination reduces the possibility of a severe course of the disease.

AIM

The aim of this study is to investigate whether the vaccination status of patients hospitalized due to COVID-19 has an effect on the CT severity score (CT-SS) and CORADS score obtained during hospitalization.

METHODS

The files of patients hospitalized between April 1, 2021 and April 1, 2022 due to COVID-19 were retrospectively reviewed. A total of 224 patients who were older than 18 years of age, whose vaccination status was accessible, whose severe acute respiratory syndrome coronavirus 2 polymerase chain reaction result was positive, and who had a Thorax CT scan during hospitalization were included in the study.

RESULTS
Among the patients included in the study, 52.2% were female and the mean age was 61.85 years. The patients applied to the hospital on the average 7th day of their complaints. While 63 patients were unvaccinated (Group 1), 20 were vaccinated with a single dose of CoronaVac (Group 2), 24 with a single dose of BioNTech (Group 3), 38 with 2 doses of CoronaVac (Group 4), 40 with 2 doses of BioNTech (Group 5), and 39 with 3 doses of vaccine (2 doses of CoronaVac followed by a single dose of BioNTech, Group 6). CT-SS ranged from 5 to 23, with a mean of 12.17.

RESULTS
CT-SS mean of the groups were determined as 14.17, 13.35, 11.58, 10.87, 11.28, 10.85, respectively. Accordingly, as a result of the comparisons between the groups, the CT-SS levels of the unvaccinated patients found to be significantly higher than the other groups. As the vaccination rates increased, the rate of typical COVID-19 findings on CT was found to be significantly lower.

CONCLUSION
Increased vaccination rates in COVID-19 patients reduce the probability of typical COVID-19 symptoms in the lungs. It also reduces the risk of severe disease and decreases CT Severity Scores. This may lead to a loss of importance of Thorax CT in the diagnosis of COVID-19 pneumonia as the end of the pandemic approaches.

Key Words: COVID-19; CORADS; Computed tomography severity score; Thorax computed tomography; SARS-CoV-2; Vaccination

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Core Tip: This is a retrospective study to evaluate the effect of vaccination status on CORADS and computed tomography (CT) severity score in hospitalized coronavirus disease 2019 (COVID-19) patients. Accordingly, as a result of the comparisons between the groups, the CT severity score levels of the unvaccinated patients were significantly higher than the other groups. As the vaccination rates increased, the rate of typical COVID-19 findings on CT was found to be significantly lower.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic still continues, although the number of cases has decreased[1]. The disease most commonly affects the lungs, and since the beginning of the pandemic, thorax computed tomography (CT) has been an indispensable imaging method for diagnosis and follow-up[2]. The disease is tried to be controlled with the introduction of vaccines and the increase in vaccination rates. Vaccination reduces the probability of severe course of the disease and the CORADS score, which is an indicator of lung involvement[3-6].

CoronaVac vaccine (Sinovac Life Sciences, Beijing, China), which is an inactive vaccine, has been started to use from the elderly population and healthcare workers in our country as of January 2021[7]. As of April 2021, the BNT-162b2 (BioNTech/Pfizer) vaccine, which is an mRNA vaccine, has begun to be used[8]. Reminder doses are also applied as the pandemic continues.

The aim of this study was to investigate whether the vaccination status of patients hospitalized due to COVID-19 had any effect on the CT severity score (CT-SS) and CORADS scoring assessed during hospitalization.

MATERIALS AND METHODS

Study design

This study was planned as a retrospective, cross-sectional study and was conducted with the approval of Erzincan Binali Yildirim University Clinical Research Ethics Committee (Date: 27.10.2022 / Decision No: 04/16).

The files of patients admitted to the COVID-19 inpatient clinic between April 1, 2021 and April 1, 2022 were retrospectively reviewed.

Inclusion criteria: (1) Being over 18 years of age; (2) Having a positive polymerase chain reaction (PCR) test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and receiving inpatient treatment in one of the COVID-19 inpatient clinics; (3) Having a Thorax CT scan on the seventh day of complaints during hospitalization; (4) Having access to COVID-19 vaccination information during hospitalization; (5) Among the patients for whom vaccination information was available, it was defined as 14 days or more from the last vaccine dose for patients who received 2 or more vaccines, and 14 days or more from the date of vaccination for patients who received a single dose of vaccine.

Accordingly, 224 patients out of a total of 2000 patients were included in the study. In our study, patients who met the inclusion criteria were divided into 6 groups: Unvaccinated (Group 1), single dose CoronaVac vaccine (Group 2), single dose BioNTech vaccine (Group 3), 2 doses CoronaVac vaccine (Group 4), 2 doses BioNTech vaccine (Group 5) and 2 doses CoronaVac + 1 dose BioNTech vaccine (Group 6). Patients were evaluated according to the severity of involvement in thorax CT and whether there is typical involvement or not. At the same time, it was investigated whether the acute phase parameters of the patients were affected by the vaccination status.

Laboratory tests

The SARS-CoV-2 PCR test was studied with DS CORONEX COVID-19 Multiplex Real Time qPCR (DS Nano and Biotechnology). The hemogram test of the patients was performed using the Sysmex XN-1000 Hematology System (Sysmex Corporation, Kobe, Japan); and biochemical tests were studied with AU 5800 (Beckman Coulter, California, United States) and coagulation tests with Ceveron® alpha (Diapharma Group, Ohio, United States).

Thorax CT

All patients had a CT scan without intravenous contrast material on the day they were admitted to the hospital (Siemens SOMATOM Sensation 16, Forchheim, Germany). All patients were scanned in the supine position using an adult CT protocol; reconstruction images of the 1.5 mm lung window were obtained using tube voltage = 130kV, effective mAs = 70, slice thickness = 5 mm, collimation = 16 × 1.2, pitch = 0.8. In children, reconstruction images of the lung window of 1.5 mm were obtained with protocol tube voltage = 110kV, effective mAs = 60, slice thickness = 8 mm, collimation = 16 × 1.2, pitch = 0.8 (14 years and younger). CT-SS and CORADS score were evaluated by 3 independent experienced radiologists.

CT severity score

The result of the CT examination on admission to hospital was used to define the CT severity value. CT severity of the patients was defined for each lung segment, and the sum of the severity value of each lobe was used to arrive at a final severity score. CT severity scores were calculated using the method described by Pan *et al*[9] (Table 1). The mean value of two measurements was recorded as the final value.

CORADS score

The CORADS system was used to determine the probability of disease based on the severity of lung involvement in CT examinations performed during hospitalization. CORADS 0 indicates that the examination is not of sufficient quality to be evaluated. CORADS 1 indicates either a normal thorax scan or the presence of a non-infectious disease. CORADS 2 identifies findings that are unusual for COVID-19 but are common in other infectious diseases such as bronchitis and bronchopneumonia. CORADS 3 describes findings that could be related to COVID 19 as well as other viral pneumonias or non-infectious diseases. CORADS 4 identifies findings that are typical for COVID 19 but may also be relevant for other viral pneumonias. Its difference from CORADS 5 is its atypical involvement. CORADS 5 category implies a very high level of suspicion for pulmonary involvement by COVID-19. Patients who are positive according to the reverse transcription-PCR test result are defined as CORADS 6[10].

Statistical analysis

NCSS (Number Cruncher Statistical System) 2020 (Kaysville, Utah, United States) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, maximum) were used while evaluating the study data. The suitability of the quantitative data to the normal distribution was tested by the Shapiro-Wilk test and graphical analyses.

Oneway ANOVA test was used for the comparison of normally distributed quantitative variables between groups, and Bonferroni test was used for post hoc evaluations. The Kruskal-Wallis test was used for the comparison of quantitative variables that did not show normal distribution, and the Dunn-Bonferroni test was used for post hoc evaluations.

Pearson Chi-Square test was used to compare qualitative data. Statistical significance was accepted as $P < 0.05$.

RESULTS

The study was conducted in a university hospital from the eastern of Turkey between April 1, 2021 and April 1, 2022, with a total of 224 cases, of which 52.2% (n = 117) were female and 47.8% (n = 107) were male. The ages of the cases ranged from 22 to 97, and the mean age was 61.85±15.36 years.

A statistically significant difference was found between the age distributions of the groups ($P < 0.001$); when the significances are analyzed; the mean age of those who were not vaccinated was found to be significantly lower than the single dose CoronaVac, two doses CoronaVac, two doses BioNTech and three doses vaccine groups ($P = 0.021$; $P = 0.001$; $P = 0.011$; $P = 0.001$; $P < 0.05$, respectively).

Table 1 Computed tomography severity scoring

CT severity score	Extent of lesions for each lung lobe, %
0	0
1	< 5
2	5-25
3	26-50
4	51-75
5	> 75

CT: Computed tomography.

The mean age of those who received a single dose of CoronaVac was higher than those who received a single dose of BioNTech ($P = 0.049$), and significantly lower than the two-dose CoronaVac and three-dose vaccine groups ($P = 0.002$; $P = 0.001$; $P < 0.01$, respectively).

The mean age of those who received a single dose of BioNTech was also found to be significantly lower than the two-dose CoronaVac and three-dose vaccine groups ($P = 0.001$; $P = 0.001$; $P < 0.01$, respectively).

The ages of those who received two doses of CoronaVac were also significantly higher than those who received two doses of BioNTech ($P = 0.001$; $P < 0.01$). The age of those who received two doses of BioNTech was also significantly lower than the group that received three doses of vaccine ($P = 0.001$; $P < 0.01$).

No statistically significant difference was found between the distribution of the groups according to gender ($P > 0.05$) (Table 2).

A statistically significant difference was found between the CT severity scores according to the groups ($P < 0.01$). When it was analyzed from which groups the significance originated, no significant difference was found between the severity scores of those who were not vaccinated and those who received a single dose of CoronaVac and a single dose of BioNTech ($P > 0.05$). The severity score was significantly higher than those who received two doses of CoronaVac, two doses of BioNTech, and three doses of vaccination ($P < 0.05$) (Table 3, Figure 1).

A statistically significant difference was found between the distribution of the groups according to the CORADS classification according to their typicality ($P = 0.001$; $P < 0.01$). When the significances were analyzed, the typical incidence rate in those who were not vaccinated and those who received a single dose of CoronaVac vaccine was significantly higher than in all other groups. Typical rates of those who received a single dose of BioNTech and those who received two doses of CoronaVac vaccine were also found to be higher than those who received two doses of BioNTech and three doses of vaccine (Table 4).

A statistically significant difference was found between the C-reactive protein (CRP) levels of the groups ($P < 0.01$). When it was analyzed from which groups the significance originated, CRP levels of those who received a single dose of BioNTech vaccine were found to be statistically significantly lower than those who received two doses of CoronaVac, two doses of BioNTech, and three doses of vaccine ($P = 0.012$; $P = 0.014$; $P = 0.008$; $P < 0.05$). No significant difference was found between the CRP levels of the other groups ($P > 0.05$) (Table 5, Figure 2).

A statistically significant difference was found between the lactate dehydrogenase (LDH) levels of the groups ($P < 0.01$). When it was analyzed from which groups the significance originated, LDH levels of those who received three doses of vaccine, two doses of CoronaVac vaccine, one dose of BioNTech, and those who were not vaccinated were statistically significantly lower ($P = 0.010$; $P = 0.038$; $P = 0.001$; $P < 0.05$). The LDH levels of those who received two doses of BioNTech were significantly lower than those who were not vaccinated ($P = 0.040$; $P < 0.05$). No significant difference was found between the LDH levels of the other groups ($P > 0.05$) (Table 5, Figure 3).

There was no statistically significant difference between the lymphocyte levels of the groups ($P > 0.05$) (Table 5).

A statistically significant difference was found between the procalcitonin levels of the groups ($P < 0.01$). It was analyzed from which groups the significance originated, the procalcitonin levels of those who received a single dose of BioNTech were found to be statistically significantly lower than those who received a single dose of CoronaVac vaccine, two doses of CoronaVac and two doses of BioNTech ($P = 0.001$; $P = 0.001$; $P = 0.042$; $P = 0.037$; $P <0.05$). No significant difference was found between the Procalcitonin levels of the other groups ($P > 0.05$) (Table 5, Figure 4).

There was no statistically significant difference between D-Dimer levels according to the groups ($P > 0.05$) (Table 5).

DISCUSSION

In our country, COVID-19 vaccination was started with the CoronaVac vaccine, which is an inactive vaccine. The vaccine was first started to be administered to healthcare workers and elderly individuals, and vaccination continued from older individuals to younger individuals gradually. While the pandemic was continuing, the BNT-162b2 (BioNTech) vaccine, which is an mRNA vaccine, has also been started to be used in our country[7,8]. This explains why the average age of those who received two doses of CoronaVac and three doses of vaccine in our study was higher.

Table 2 Evaluations of the groups by age and gender

	n	Age	Gender	
		mean ± SD	Male	Female
[1]Unvaccinated	63	50.95 ± 13.54	27 (42.9)	36 (57.1)
[2]Single dose CoronaVac	20	60.75 ± 8.82	9 (45.0)	11 (55.0)
[3]Single dose BioNTech	24	50.1 ± 11.015	7 (29.2)	17 (70.8)
[4]Two doses CoronaVac	38	73.55 ± 9.34	21 (55.3)	17 (44.4)
[5]Two doses BioNTech	40	59.13 ± 13.88	24 (60)	16 (40)
[6]Three doses	39	74.23 ± 10.29	19 (48.7)	20 (51.3)
Total	224	61.09 ± 15.36	107 (47.8)	117 (52.0)
P value		0.001[a,c]	0.204[b]	
Post hoc		1 < 2, 4, 5, 6; 2 > 3; 2 < 4, 6; 3 < 4, 6; 4 > 5; 5 < 6	-	

[a]Oneway Anova test.
[b]Pearson chi-square test.
[c]$P < 0.01$.

Table 3 Evaluation of computed tomography severity scores of the groups

Group	*n*	CT severity scores				*P* value
		Mean	SD	Median	Min-Max	
[1]Unvaccinated	63	14.17	4.56	15	5-23	0.002[b]
[2]Single dose CoronaVac	20	13.35	4.12	13.5	5-21	
[3]Single dose BioNTech	24	11.58	5.29	11.5	5-20	1 > 4; $P = 0.015$[a]
[4]Two doses CoronaVac	38	10.87	4.42	10.5	5-19	1 > 5; $P = 0.021$[a]
[5]Two doses BioNTech	40	11.28	5.25	12.0	5-21	1 > 6; $P = 0.045$[a]
[6]Three doses	39	10.85	5.01	9	5-22	
Total	224	12.17	4.950	12	5-23	

Kruskal Wallis test & post hoc Dunn test.
[a]$P < 0.05$.
[b]$P < 0.01$. CT: Computed tomography.

Thorax CT has been used since the beginning of the pandemic to diagnose COVID-19 suspected patients, especially those with negative SARS-CoV-2 PCR tests. With the CORADS scoring system, patients are evaluated for COVID-19 with Thorax CT. At the same time, Thorax CT is used for disease severity scoring[11,12]. The pandemic is tried to be controlled with the introduction of COVID-19 vaccines. In the study of Haas *et al*[13], it was shown that 2 doses of BioNTech vaccine reduced the risk of severe disease and death. Hu *et al*[14] also found that 2 doses of inactive vaccine were highly effective against the Delta variant and reduced the risk of severe disease. In the study of Sagiraju *et al*., it was shown that full dose vaccination reduces the risk of severe disease and mortality, regardless of the vaccine type[15]. In the study of Mahajan *et al*[16], where they evaluated vaccinated and unvaccinated patients with a positive SARS-CoV-2 PCR test, they found that the CT-SS of vaccinated people was mild-moderate, while unvaccinated individuals were moderate-severe. Again, in the studies conducted by Russo *et al*[4], Ravindra *et al*[5], Fatima *et al*[6], and Yavuz *et al*[17], when the CT SS of vaccinated and unvaccinated patients were compared, it was shown that vaccinated patients had milder pulmonary involvement. In our study, it was shown that unvaccinated individuals had moderate-severe lung involvement, and patients who received two doses of CoronaVac or two doses of BioNTech or three doses of vaccine had moderate lung involvement. Also, it was shown that incomplete vaccination was not effective in reducing CT SS. This finding supports the literature. At the same time, it is an indication that incomplete vaccination will not have any effect on severe disease.

In our study, unlike other studies, the group that had a reminder dose with mRNA vaccine after 2 inactivated vaccines was also evaluated. Accordingly, the CT SS of the patients who received 3 doses of vaccine was found to be lower than those who received 2 doses of vaccine, although it was not statistically significant. This is an evidence of the necessity of reminder doses.

Table 4 Evaluation of groups according to CORADS classification

Group	*n*	CORADS		*P* value
		Typical	Not typical	
[1]Unvaccinated	63	57 (90.5)	6 (9.5)	0.001[a]
[2]Single dose CoronaVac	20	18 (90)	2 (10.3)	
[3]Single dose BioNTech	24	14 (58.3)	10 (41.7)	
[4]Two doses CoronaVac	38	26 (68.4)	12 (31.6)	
[5]Two doses BioNTech	40	15 (37.5)	25 (62.5)	
[6]Three doses	39	17 (43.6)	22 (56.4)	
Total	224	147 (65.6)	77 (34.4)	

[a]$P < 0.01$. Pearson chi-square test.

Table 5 Distribution of Acute Phase Variables by Groups

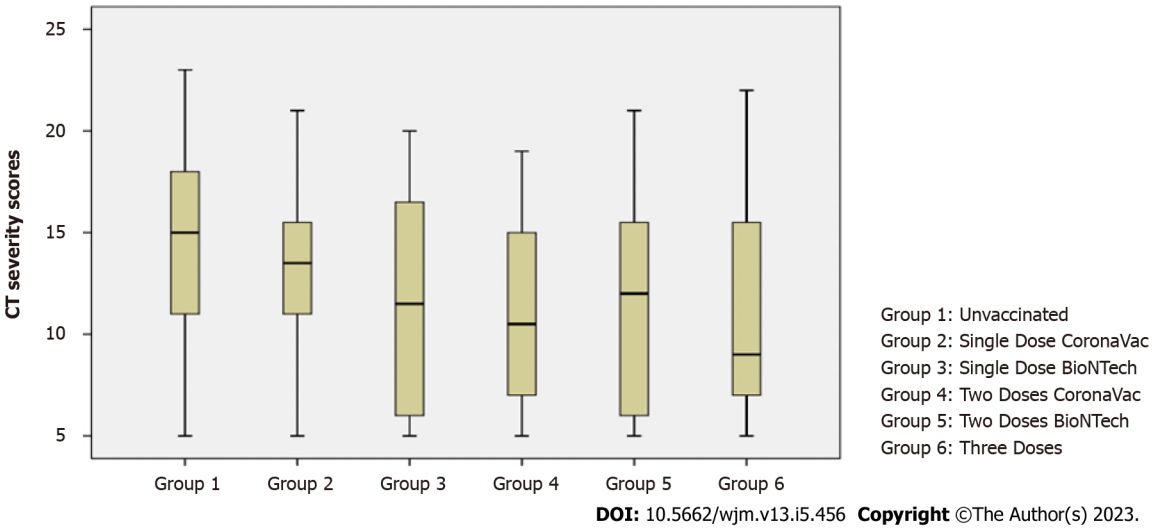
		[1]Unvaccinated	[2]Single dose CoronoVac	[3]Single dose BioNTech	[4]Two doses CoronaVac	[5]Two doses BioNTech	[6]Three doses	*P* value[c]	Post Hoc
CRP	mean ± SD	52.95 ± 40.49	49.75 ± 31.26	34.0 ± 32.3	66.58 ± 52.75	84.35 ± 72.99	81 ± 62.88	0.006[a]	3 < 4, 5, 6
	Median (Min-Max)	46 (3-173)	39.5 (12-124)	22 (2-106)	59 (4-204)	66 (2-341)	54 (2-286)		
LDH	mean ± SD	359.17 ± 164.41	315.2 ± 58.2	331.83 ± 116.61	401.5 ± 305.74	294.05 ± 89.57	276.36 ± 94.2	0.001[a]	6 < 5, 3, 1; 5 < 1
	Median (Min-Max)	359 (164-1213)	311 (195-461)	328 (207-692)	368 (156-1943)	290 (152-640)	273 (131-538)		
Lymphocyte count	mean ± SD	1335.4 ± 512.04	1396.5 ± 470.93	1502.92 ± 724.42	1637.11 ± 1896.86	1628.75 ± 86.02	1477.95 ± 867.82	0.660	
	Median (Min-Max)	1290 (390-2790)	1375 (510-2050)	1340 (420-3450)	1200 (340-12360)	1575 (550-5200)	1320 (260-4640)		
Procalcitonin	mean ± SD	0.52 ± 0.94	0.28 ± 0.29	0.13 ± 0.13	0.77 ± 2.39	0.25 ± 0.2	0.27 ± 0.31	0.001[a]	3 < 1, 2, 4, 5
	Median (Min-Max)	0.3 (0.1-6)	0.3 (0.1-1.3)	0.1 (0.1-0.7)	0.2 (0.1-15)	0.1 (0.1-0.7)	0.1 (0.1-1.2)		
D-DIMER	mean ± SD	1041.08 ± 1559.79	955.95 ± 1156.16	1396.71 ± 1608.97	1561.63 ± 2118.83	1462.2 ± 2934.47	1388.41 ± 1922.45	0.106	
	Median (Min-Max)	596 (24-11805)	549.5 (133-4661)	714.5 (140-6193)	942.5 (251-13077)	622 (166-17459)	820 (194-9440)		

[a]$P < 0.01$.

[c]Kruskal Wallis test & post hoc Dunn test.

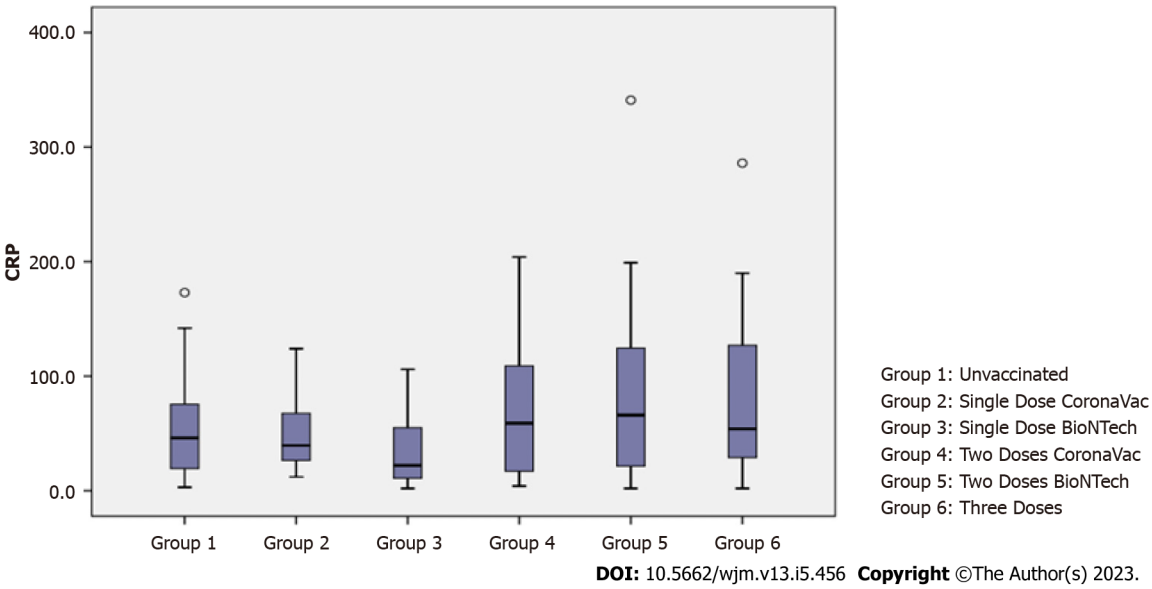
Since the beginning of the pandemic, Thorax CT has had an important place in the diagnosis of suspected COVID-19 patients, especially those with negative PCR tests. Patients with negative SARS-CoV-2 PCR test were not included in our study, and patients were admitted to the hospital on the 7th day of their symptoms on average. When the CORADS scores of the diagnosed cases were evaluated, it was found that the typical appearance was significantly less as the number of vaccinations increased. This suggests that while the pandemic continues, the role of CT in the diagnosis of COVID-19 may remain in the background.

In a study evaluating the effectiveness of the CoronaVac vaccine and hospitalizing 292 patients diagnosed with COVID-19, no significant difference was found between the acute phase reactants of vaccinated and unvaccinated patients[17]. In another study conducted in Poland, no significant difference was found between acute phase reactants [18]. In our study, different acute phase reactants (CRP and procalcitonin) responses were observed depending on the number and type of vaccination, and it was thought that it could not be interpreted clearly since only the acute phase reactants of the patients at the time of admission to the hospital were examined. Otherwise, LDH level is associated with the prognosis of the disease[19]. In our study, the higher LDH levels of unvaccinated or incompletely vaccinated patients showed that vaccination would reduce the probability of a severe course of the disease but, only the measurements of the



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Figure 1 Distribution of computed tomography severity scores of the groups. CT: Computed tomography.



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Figure 2 Distribution of C-reactive protein levels of the groups. CRP: C-reactive protein.

patients at the time of admission to the hospital were evaluated in our study.

Strength of the study

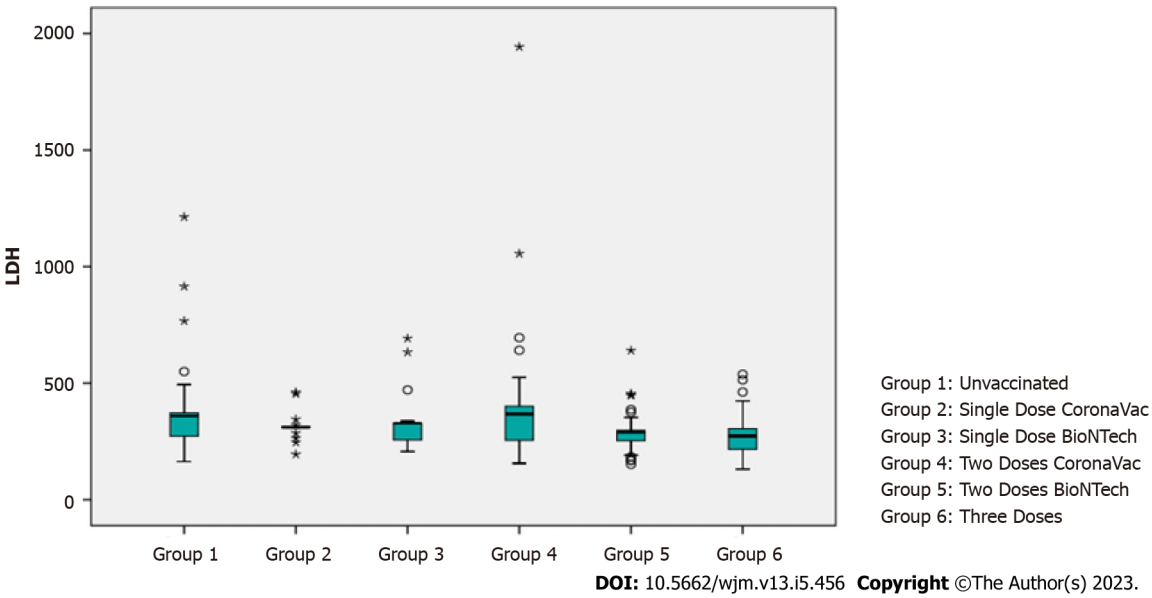
The strength of our study is that, unlike other studies, we evaluated the group that received a booster dose with mRNA vaccine after 2 doses of inactive vaccine.

Limitations of the study

The acute phase reactants in the follow-up of the patients were not evaluated. This is one of the limitations of the study. Again, not evaluating the comorbid diseases and mortality rates of the patients are other limitations of our study.

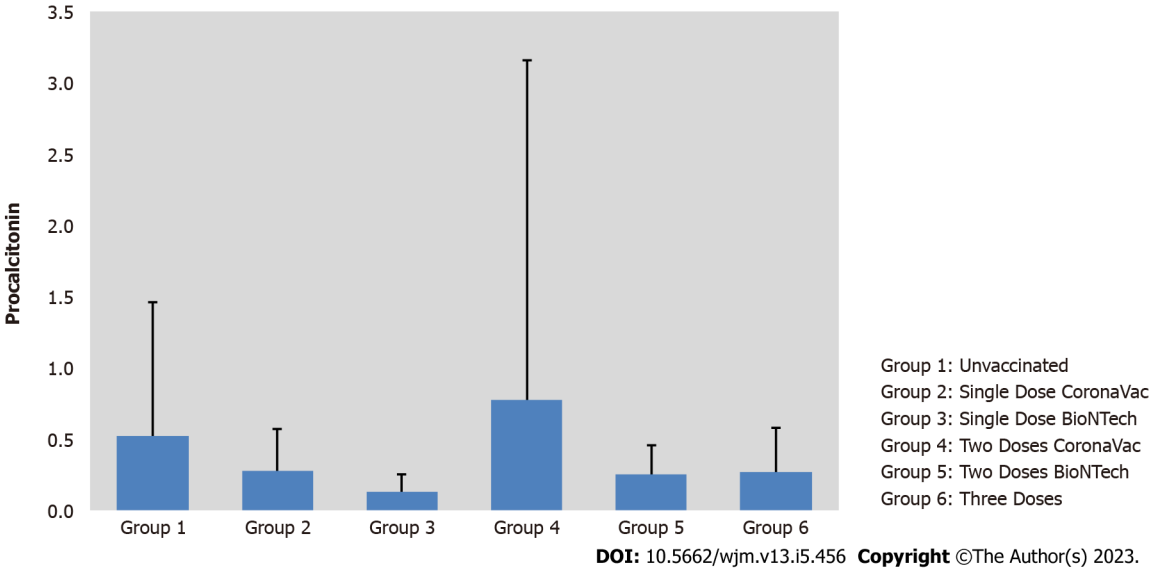
CONCLUSION

In conclusion, our study showed that being vaccinated, regardless of the type of vaccine, leads to a decrease in CT severity scores. Again, our study suggests that with the effect of vaccination, we may no longer see the CT images that we are accustomed to seeing in COVID-19 patients, and CT may remain in the background in diagnosis.



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Figure 3 Distribution of lactate dehydrogenase levels of the groups. LDH: Lactate dehydrogenase.



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Figure 4 Distribution of procalcitonin levels of the groups.

ARTICLE HIGHLIGHTS

Research background

The coronavirus disease 2019 (COVID-19) pandemic is still continuing. Vaccination has an important place in preventing the disease and vaccination reduces the possibility of a severe course of the disease.

Research motivation

It is important to investigate whether vaccination has any effect on the computed tomography (CT) severity score (CT-SS) and CORADS score of COVID-19 patients.

Research objectives

We aim to investigate whether the vaccination status of inpatient treatment for COVID-19 has any effect on the CT-SS and CORADS score taken during hospitalization.

Research methods

This single-center retrospective study was conducted between April 1, 2021 and April 1, 2022 with a total of 224 patients older than 18 years of age, whose vaccination status was accessible, who had positive severe acute respiratory syndrome

coronavirus 2 polymerase chain reaction results, and who had a thorax CT taken during hospitalization.

Research results

Among the patients included in the study, 52.2% were female and the mean age was 61.85 years. The patients applied to the hospital on the average 7th day of their complaints. While 63 patients were unvaccinated (Group 1), 20 were vaccinated with a single dose of CoronaVac (Group 2), 24 with a single dose of BioNTech (Group 3), 38 with 2 doses of CoronaVac (Group 4), 40 with 2 doses of BioNTech (Group 5), and 39 with 3 doses of vaccine (2 doses of CoronaVac followed by a single dose of BioNTech, Group 6). CT-SS ranged from 5 to 23, with a mean of 12.17. CT-SS mean of the groups were determined as 14.17, 13.35, 11.58, 10.87, 11.28, 10.85, respectively. Accordingly, as a result of the comparisons between the groups, the CT-SS levels of the unvaccinated patients found to be significantly higher than the other groups. As the vaccination rates increased, the rate of typical COVID-19 findings on CT was found to be significantly lower.

Research conclusions

The increase in vaccination rates in COVID-19 patients reduces the CT Severity Score and the CORADS score.

Research perspectives

It is important that COVID-19 vaccinations continue. With the effect of vaccination, the possibility of severe course of the disease will decrease. There will be a need for studies in which more patient data are analyzed and data obtained from patients with different vaccines are evaluated.

FOOTNOTES

Author contributions: Binay UD designed and performed the research and wrote the manuscript; Karavas E designed the research and supervised the report; Karakeçili F designed the research and contributed to the analysis; Barkay O, Aydin S, Senbil DC provided clinical advice; Barkay O, Aydin S, Senbil DC supervised the report. All authors have read and approved the final manuscript.

Institutional review board statement: This study was reviewed and approved by the Erzincan Binali Yildirim University Ethics Committee (Approval No: 04/16).

Informed consent statement: Since it is a retrospective study, patient consent was not obtained.

Conflict-of-interest statement: All the authors declare that they have no conflict of interest.

Data sharing statement: Data will be shared upon request.

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Country/Territory of origin: Turkey

ORCID number: Umut Devrim Binay 0000-0003-3841-9109; Erdal Karavaş 0000-0001-6649-3256; Faruk Karakeçili 0000-0002-7368-7187; Orçun Barkay 0000-0001-5347-7740; Sonay Aydin 0000-0002-3812-6333; Düzgün Can Şenbil 0000-0003-0233-7371.

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ORIGINAL ARTICLE

Observational Study

Study on good clinical practices among researchers in a tertiary healthcare institute in India

Harshita Harshita, Prasan Kumar Panda

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Harshita Harshita, Medical School, All India Institute of Medical Sciences, Rishikesh 249203, Uttarakhand, India

Prasan Kumar Panda, Department of Medicine, All India Institute of Medical Sciences, Rishikesh 249203, Uttarakhand, India

Prasan Kumar Panda, Department of Medicine, College Block, All India Institute of Medical Sciences, Rishikesh 249203, Uttarakhand, India

Corresponding author: Prasan Kumar Panda, MBBS, MD, Associate Professor, Department of Medicine, All India Institute of Medical Sciences, Room No. 409, Rishikesh 249203, Uttarakhand, India. motherprasanna@rediffmail.com

Abstract

BACKGROUND

Good clinical practice (GCP) is put in place to protect human participants in clinical trials as well as to ensure the quality of research. Non-adherence to these guidelines can produce research that may not meet the standards set by the scientific community. Therefore, it must be ensured that researchers are well-versed in the GCP. But not much is known about the knowledge and practices of the GCP in the medical colleges of North India.

AIM

To assess the knowledge and practices of researchers about GCP and analyze these with respect to the demographics of participants.

METHODS

This is a cross-sectional study. A self-structured questionnaire about GCP, after expert validations, was circulated among researchers, at a tertiary healthcare institute, All India Institute of Medical Sciences (AIIMS), Rishikesh. A total of 59 individuals, who were selected by universal sampling, participated in the study. All healthcare workers who have been investigators of Institutional Ethics Committee-approved research projects, except residents and faculty, and are still a part of the institute have been included in the study. The study was approved by the Institutional Ethics Committee of AIIMS, Rishikesh. We used descriptive analysis and the Chi-squared test to analyze data. P value < 0.05 was considered significant.

RESULTS

Out of 59 participants, only 11 (18.6%) were certified for GCP. Most of the participants (64.4%) had "Average" knowledge, 33.9% had "Good" knowledge and 1.7% had "Poor" knowledge. Only 49% of participants had satisfactory practices related to GCP. There was a significant difference in the knowledge based on the current academic position for the items assessing knowledge of institutional review board (P = 0.010), confidentiality & privacy (P = 0.011), and participant safety & adverse events (P < 0.001). There was also a significant difference in knowledge of research misconduct (P = 0.024) and participant safety & adverse events (P = 0.011) based on certification of GCP. There was a notable difference in the practices related to recruitment & retention on the basis of current academic position (P < 0.001) and certification of GCP (P = 0.023). We also observed a considerable difference between the knowledge and practices of GCP among the participants (P = 0.013).

CONCLUSION

Participants have basic knowledge of GCP but show a lack thereof in certain domains of GCP. This can be addressed by holding training sessions focusing on these particular domains.

Key Words: Clinical trial; Ethics; Good clinical practice; Knowledge; Research; Research subjects

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Core Tip: There is a lack of knowledge about the good clinical practices (GCP) in the researchers of medical colleges. In order to improve the quality of research, as well as, make research a better experience for the participants of research, we must work on improving awareness of the GCP among researchers. This can be done by organising training sessions or workshops which throw light on the principles of GCP.

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INTRODUCTION

With the increase in research studies involving human subjects, there arises a need to have certain guidelines in place to protect human subjects. Additionally, the review of research by the research ethics committee (REC) has been mandated by international standards[1,2]. A lack of guidelines can lead to misuse of participants as well as other resources. Good clinical practice (GCP) provided by the International Conference on Harmonization (ICH) sets ethical and scientific standards and guidelines for conducting research involving human participants[3]. The two important principles of these guidelines include protecting the rights of human participants and the credibility of the data generated[4]. Such guidelines are required for the welfare of individuals partaking in a trial.

Knowledge of GCP before taking on a research project would lead to increased safety and efficacy of the project. A study conducted in Saudi Arabia, in which 85% of respondents had been trained for GCP, estimated that 97% of respondents believed that GCP guidelines were followed in trials and they improved the quality of the trial. While 59% of the respondents were cynical towards the institutional review board (IRB) approval process or the monitoring of the clinical trials[5]. Another study, conducted among dental faculties in India, established that more than 93% of the respondents wanted research ethics education for post-graduates, principal investigators as well as members of the REC, while less than 20% thought that the REC was not needed because of the presence of scientific committee[6].

The objective of our study was to estimate the knowledge and practices of researchers in a tertiary care institute in Northen-India. We also want to determine the difference in knowledge and practices based on research experience, GCP certification, and current academic position in the institute. Our null hypothesis stated that there is no knowledge/practice gap of GCP among researchers in a tertiary care research institution.

MATERIALS AND METHODS

Study Design

We conducted a cross-sectional study, which used a self-administered questionnaire at the All India Institute of Medical Sciences, Rishikesh between February and April 2023.

Study Population

We recruited all the researchers who had Institutional Ethics Committee-approved research projects. Here, the individuals, other than faculty and residents, who have conducted research in the institute are referred to as researchers.

Questionnaire

The questionnaire was self-structured and based on the GCP guidelines[7]. The study tool included 4 sections. The first section contained a summary of the study and informed consent for participation in the study. This was followed by section 2, which had questions that characterized the demographics of the participants. This included age, sex, experience in research, current academic position, number of publications, and certification for GCP. The third section consisted of 13 questions that assessed the knowledge of participants regarding GCP guidelines. Three of these questions were evaluated by a Likert scale (1- strongly agree, 2- agree, 3- neutral, 4- disagree, 5- strongly disagree), 9 were True/False questions and 1 was a Yes/No question. The last section assessed the application of GCP principles by the participants in practice through 10 case scenarios. In these, 4 options were given out of which one had to be chosen by the participants. Questions were based on the course of GCP by National Institute on Drug Abuse (NIDA) Clinical Trials Network and references were taken from previous studies[5,7].

All 23 items assessing the Knowledge and practices of participants were categorized into 1 of the 12 modules of NIDA Clinical Trials Network's course of GCP: Introduction (n = 2), IRB (n = 2), informed consent (n = 2), confidentiality & privacy (n = 4), participant safety & adverse events (n = 3), quality assurance (n = 2), the research protocol (n = 1), documentation & record-keeping (n = 0), research misconduct (n = 4), roles & responsibilities (n = 0), recruitment & retention (n = 2), investigational new drugs (n = 1)[7].

The questionnaire was subjected to content validation by researchers well versed in the GCP guidelines and modifications were made according to their suggestions.

The questionnaire was sent to the participants as Google forms and it was ensured that the participants fully understand the items before responding to them. To ensure quality, the responses were verified and screened for non-response/invalid responses.

Statistics

Data analysis was done using Jamovi software. We report the positive responses for the items in mean scores.

For True/False and Yes/No items, a score of 1 was given to the correct response and 0 to the incorrect response. For the Likert scale, a score of 0 was given to the most negative response and a score of 4 to the most positive response. The composite score for knowledge was calculated by adding the scores of all 13 items, the maximum possible score was 22. As for the practice-based questions, a score of 1 was given to the correct answer and a score of 0 was given if the participant chose any other options. The total score was calculated by simple summation of the scores of all 10 items (maximum score = 10). The knowledge was classified as "good" (score > 16), "average" (score = 11-16), and "poor" (score < 11). Similarly, the practices of the participants were classified as "satisfactory" (score ≥ 7) and "unsatisfactory" (score < 7).

Demographic data were described using descriptive statistics. Chi-squared test in the bi-variate analysis was used to determine the relationship between knowledge and demographic details like certification of GCP, current academic position, and experience in research. The same was done to determine the association between practices of GCP and independent variables of demographics. To determine the association of knowledge and practices of GCP, a chi-square test was used. $P < 0.05$ was considered significant.

Ethical Consideration

The approval to conduct this research was taken from the Institutional Ethics Committee of All India Institute of Medical Sciences, Rishikesh, India.

Informed consent, which was subjected to the approval of the Institutional Ethics Committee, was taken from all the individuals before enrolling them in the study.

In order to maintain privacy, any data which may have identified the individual participants of the study was not disclosed.

RESULTS

A total of 135 individuals were contacted, out of which 67 consented to fill out the questionnaire and 59 responses were valid. Table 1 shows the demographic details of the participants. The age of the respondents ranged from 19 years to 38 years, with a mean age of 23 years and a median of 22 years. Most of the participants were Bachelor of Medicine, Bachelor of Surgery students (66.1%). Only 10.2% of participants had more than 3 years of research experience while a majority (57.6%) had less than 1 year of experience in conducting a research. Eighteen individuals (30.5%) had research publications. Out of the respondents, 25.4% have been resource persons for academic classes on research methodologies.

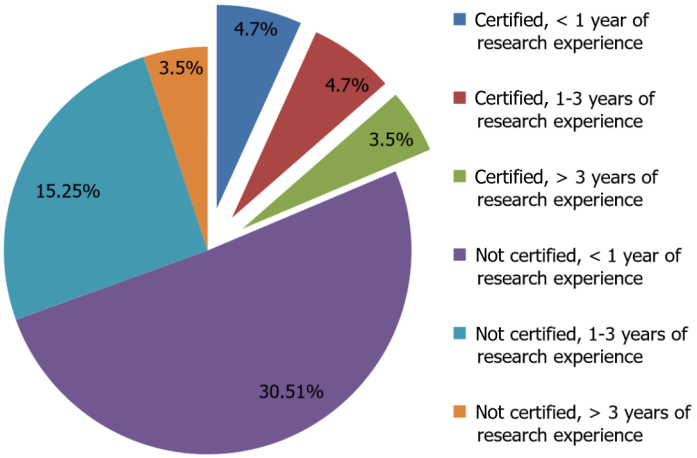
The relationship between research experience and certification of GCP has been shown in Figure 1.

The participants' knowledge was assessed by 13 items, of which 10 were "Yes/No" or "True/False" items and 3 used the Likert scale. Knowledge scores in our sample ranged from a minimum of 10 (45% correct) to a maximum of 18 (82% correct). The mean score was 15.4 (out of 22) with a standard deviation of 1.95. Knowledge was deemed "Good" if the score was more than equal to 17, "Average" if the score was from 11 to 16, and "Poor" if the score was less than 11.

Table 1 Demographics of participants

Variables		Number	Percent (%)
Sex	Female	25	42.4
	Male	34	57.6
Age	< 25 years	47	79.7
	≥ 25 years	12	20.3
Academic qualification	BSc	8	13.6
	MBBS	39	66.1
	MPH	3	5.1
	MSc	6	10.2
	PhD	3	5.1
Total research experience	< 1 year	34	57.6
	1-3 years	19	32.2
	> 3 years	6	10.2
Number of publications	Not yet published	41	69.5
	1-3	15	25.4
	> 3	3	5.1
Have been a resource person for academic classes on research methodologies	Yes	15	25.4
	No	44	74.6
Certified for GCP course	Yes	11	18.6
	No	48	81.4

BSc: Bachelor of science; MBBS: Bachelor of medicine, Bachelor of Surgery; MPH: Master of public health; MSc: Master of science; PhD: Doctor of philosophy; GCP: Good clinical practice.

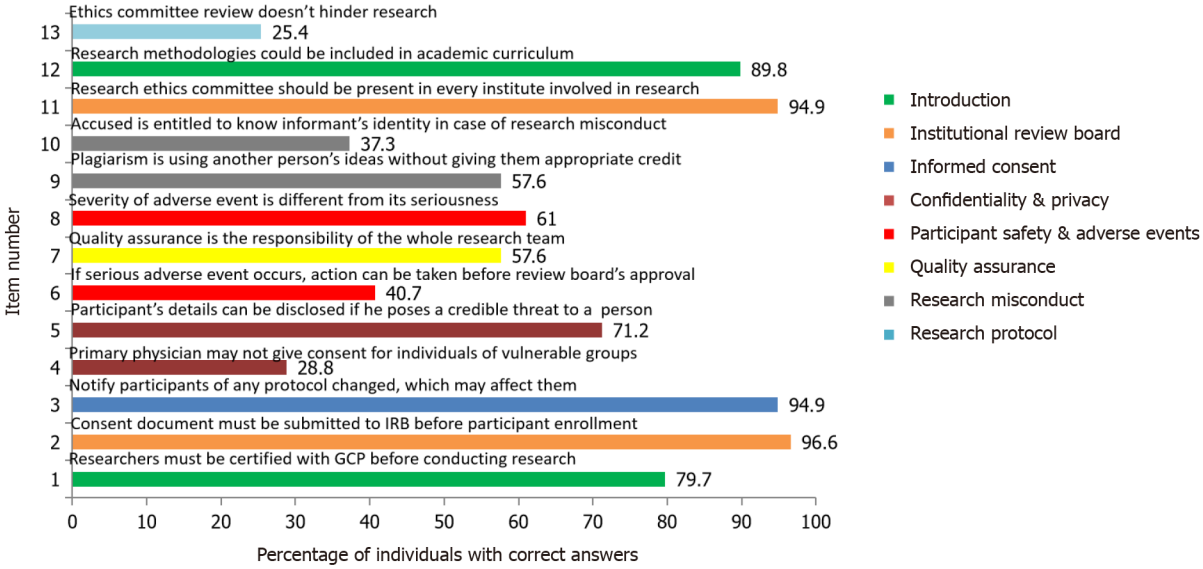


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Figure 1 Research experience with respect to certification of good clinical practice.

As seen in Figure 2, the maximum number of correct answers were given for the statement "A consent document must be submitted to the Institutional Review Board for its approval before enrolling participants in the study." (Item No. 2) with 96.6% correct responses. The statement with the maximum number of incorrect answers was "Review by the ethics committee is time consuming and makes it difficult to conduct research." (Item No. 13) which had 25.4% correct answers.

There was a significant difference in the response of the participants on the basis of current academic position for the statements "A consent document must be submitted to the IRB for their approval before enrolling participants in the study." (P = 0.010), "Participant's information can be disclosed if he/she makes a credible threat to harm another person." (P = 0.011), "The severity of an adverse event is same as its seriousness." (P < 0.001). We also found a considerable



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Figure 2 Module-wise arrangement of questions assessing knowledge. GCP: Good clinical practice.

difference on the basis of certification of GCP for the following statements: "The severity of an adverse event is same as its seriousness." (P = 0.011), and "Research misconduct consists of- fabrication, falsification and plagiarism. Plagiarism is using another person's ideas after giving appropriate credit." (P = 0.024). This is shown in the Supplementary Table 1.

Most of the respondents (64.4%) had "Average" knowledge and 33.9% had "Good" knowledge. No noteworthy differences in the total knowledge scores were found on the basis of duration of research experience, certification of GCP, and current academic position. However, there was a significant relationship between the practices of the participants and their academic positions in the institute. This is shown in Table 2.

Practices

The practice of the participants in accordance with GCP was assessed with the help of 10 multiple-choice questions, with a score of 1 for each correct response. Out of a total score of 10 for practice-based questions, scores more than equal to 7 were considered "Satisfactory" and scores less than 7 were considered "Unsatisfactory".

Item numbers 1, 2, and 5 which assess recruitment & retention, informed consent, and research misconduct respectively, had the maximum number of correct responses as shown in Figure 3.

As shown in Supplementary Table 2, a significant difference was found in the responses for item 3, which assesses the domain of Recruitment & Retention, based on the current academic position (P < 0.001) and certification of GCP (P = 0.023).

Only 29 participants (49.2%) had "Satisfactory" practices of GCP. Table 2 shows a significant difference in the overall practices of participants on the basis of their current academic position (P = 0.008).

Knowledge and Practices

Table 3 shows the results of a chi-squared test for independent samples, which showed a significant relationship between the knowledge and practice scores of the study participants; χ^2 (2, n = 59) = 8.62, P = 0.013.

DISCUSSION

This questionnaire-based study is the first report in North India specifically targeting knowledge and practices of GCP among researchers (excluding faculty and residents) in a medical college[5,6,8,9].

In our study, most of the researchers were not certified for GCP, which is comparable to a study by Goel *et al*[8] stating a lack of formal training for GCP in health care providers[8]. As the research experience increased, more proportion of individuals were certified with GCP. This suggests that individuals with more research experience are more likely to pursue GCP certification. Most of the respondents believed that one must be GCP certified before conducting research, despite this the scores of most of the participants in knowledge and practices were "Average". This goes on to show that individuals are aware of the GCP on the surface but do not fully understand the principles of GCP. There is a gap in knowledge and practices among participants concerning GCP. The mean score for knowledge of the participants was 70% of the maximum possible score, indicating most of the individuals had some knowledge of GCP. This differs from a study conducted in Japan which showed that ≤ 50% of nurses had knowledge about GCP[10]. Another study conducted among doctors in medical colleges in India stated a lack of knowledge of GCP[11].

Table 2 Associations between demographics, and total knowledge and practices scores

Demographics		Knowledge of good clinical practice					Practice of good clinical practice			
		Good	Average	Poor	χ^2	*P* value	Satisfactory	Unsatisfactory	χ^2	*P* value
Research experience (in years)	< 1	13	20	1	1.67	0.796	17	17	0.0357	0.982
	1-3	5	14	0			9	10		
	> 3	2	4	0			3	3		
Certified for GCP	Yes	3	7	1	4.54	0.103	3	8	2.59	0.108
	No	17	31	0			26	22		
Academic position in the institute	BSc	2	6	0	6.67	0.573	3	5	13.7	[a]0.008
	MBBS	17	21	1			23	16		
	MPH	0	3	0			3	0		
	MSc	1	5	0			0	6		
	PhD	0	3	0			0	3		

[a]$P < 0.01$.

GCP: Good clinical practice; BSc: Bachelor of science; MBBS: Bachelor of medicine, Bachelor of Surgery; MPH: Master of public health; MSc: Master of science; PhD: Doctor of philosophy.

Table 3 Association between knowledge and practices of good clinical practice

Practices of GCP	Knowledge of GCP		
	Good	Average	Poor
Satisfactory	15	14	0
Unsatisfactory	5	24	1
χ^2	8.62		
P value	[b]0.013		

[b]$P < 0.05$.

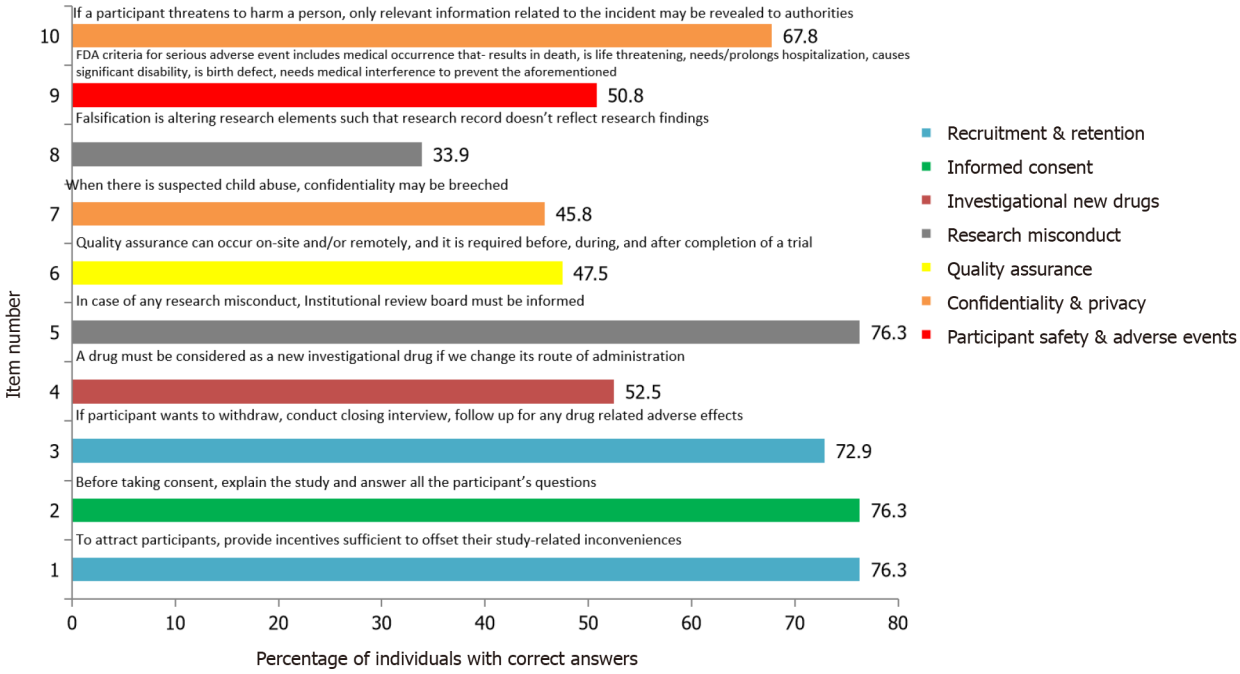
GCP: Good clinical practice.

The scores for the knowledge questions related to the topics of IRBs, and informed consent were high, indicating good knowledge of these domains. Participants obtained lower scores for questions related to confidentiality & privacy, research protocol, quality assurance, participant safety and adverse events, and research misconduct, which signifies that participants are less certain or have mixed opinions about these topics. In our study, about 74.6% of the participants believe that the review by the ethics committee is time-consuming or are unsure about this. This is similar to the finding of El-Dessouky *et al*[12], Than *et al*[9]. This delay could be perceived due to the participants' lack of understanding of the ethics committee, meticulous evaluation by the ethics committee, or due to the increased workload of the committee.

For the practice-based questions, we observed that statements related to recruitment & retention, and informed consent had a higher mean score in comparison to those related to confidentiality & privacy, quality assurance, participant safety and adverse events, research misconduct, and investigational new drugs.

We also found a significant difference between the knowledge and practices of the study participants for GCP. We also found statistical difference in the knowledge of researchers with respect to certification of GCP, or positions within the institute for items which assessed IRB, confidentiality & privacy, participant safety & adverse events, research misconduct. A noteworthy difference was found in the overall practices of GCP based on the academic positions in the institute, along with a difference in practices related to recruitment & retention for various academic positions, and certification of GCP.

At present there is no formal education about GCP in undergraduate or paramedical courses in the institute. In order to build a proper foundation of knowledge of clinical research, one must be familiar with the principles of GCP. We suggest the addition of a course on GCP in the curriculum of undergraduates and post-graduates to familiarize individuals with clinical research. Interactive training sessions can also be held, which have been shown to be effective by some studies[8,13,14].



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Figure 3 Module-wise arrangement of questions assessing practices. FDA: Food and drug administration.

Limitations

This study was not able to include the residents as well as faculty of the tertiary care institute as per the directions of the IRB. These categories include most of the researchers of the institute, thus their exclusion leading to reduced sample size as well as discrepancies between the study population and the target population. We conducted the research only in a tertiary care institute, which may limit the generalization of our results. Due to fewer participants in the study, there could be a lack in the credibility of subgroup analysis.

CONCLUSION

Our study concluded that most of the researchers (except faculty and residents) in the medical college are not certified for GCP. Individuals with more research experience are more likely to pursue GCP certification. There was a gap in the knowledge and practices of GCP among the researchers. The understanding of IRB, informed consent, and recruitment & retention was good compared to that of confidentiality & privacy, quality assurance, participant safety and adverse events, and research misconduct. There is a significant difference in knowledge or practices of individuals on the basis of GCP certification or current academic position for some domains of GCP. there is also a significant difference between the knowledge and practices of GCP. This study recommends the incorporation of GCP courses into the academic curriculum or planned training sessions for GCP for the researchers of the institute, which focus on the domains where lack of knowledge was found.

ARTICLE HIGHLIGHTS

Research background

With the increase in research studies involving human subjects, there arises a need to have certain guidelines in place to protect human subjects. Additionally, the review of research by the research ethics committee has been mandated by international standards. A lack of guidelines can lead to misuse of participants as well as other resources. Good clinical practice (GCP) provided by the international conference on harmonization sets ethical and scientific standards and guidelines for conducting research involving human participants. The two important principles of these guidelines include protecting the rights of human participants and the credibility of the data generated. The level of understanding of these GCPs among researchers is the real question to assess.

Research motivation

GCP is an essential part of research life, and assessing this will have both awareness and accountability among researchers. One method of implementation is to assess first.

Research objectives
The objective of our study was to estimate the knowledge and practices of GCP among researchers in a tertiary care institute in India.

Research methods
A self-structured questionnaire about GCP, after expert validations, was circulated among researchers, at a tertiary healthcare institute. A total of 59 individuals, who were selected by universal sampling, participated in the study. All healthcare workers who have been investigators of Institutional Ethics Committee-approved research projects, except residents and faculty, and are still a part of the institute have been included in the study. The study was approved by the Institutional Ethics Committee of All India Institute of Medical Sciences, Rishikesh. We used descriptive analysis and the Chi-squared test to analyze data. P value < 0.05 was considered significant.

Research results
Out of 59 participants, only 18.6% were certified for GCP. Sixty-four-point-four percent had "Average" knowledge, 33.9% had "Good" knowledge and 1.7% had "Poor" knowledge. Only 49% of participants had satisfactory practices related to GCP. There was a significant difference in the knowledge based on the current academic position for the items assessing knowledge of institutional review board (IRB) (P = 0.010), confidentiality & privacy (P = 0.011), and participant safety & adverse events (P < 0.001). There was also a significant difference in knowledge of research misconduct (P = 0.024) and participant safety & adverse events (P = 0.011) based on certification of GCP. There was a notable difference in the practices related to recruitment & retention on the basis of current academic position (P < 0.001) and certification of GCP (P = 0.023).

Research conclusions
This study concluded that most of the researchers (except faculty and residents) in the medical college are not certified for GCP. Individuals with more research experience are more likely to pursue GCP certification. There was a gap in the knowledge and practices of GCP among the researchers. The understanding of IRB, informed consent, and recruitment & retention was good compared to that of confidentiality & privacy, quality assurance, participant safety and adverse events, and research misconduct.

Research perspectives
There is a lack of knowledge about the GCP in the researchers of medical colleges. In order to improve the quality of research, as well as, make research a better experience for the participants of research, we must work on improving awareness of the GCP among researchers through organising training sessions or workshops which throw light on the principles of GCP.

FOOTNOTES

Author contributions: Harshita H designed, collected data, analyzed, wrote, reviewed, approved the manuscript; Panda PK gave concept, designed, analyzed, critically reviewed, and approved the manuscript.

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Informed consent statement: All study participants provided informed written consent before enrolling in the study.

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STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

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Country/Territory of origin: India

ORCID number: Harshita Harshita 0009-0006-5646-0681; Prasan Kumar Panda 0000-0002-3008-7245.

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ORIGINAL ARTICLE

Observational Study

Inflammatory bowel disease among first generation immigrants in Israel: A nationwide epi-Israeli Inflammatory Bowel Disease Research Nucleus study

Mira Stulman, Gili Focht, Yiska Loewenberg Weisband, Shira Greenfeld, Amir Ben Tov, Natan Ledderman, Eran Matz, Ora Paltiel, Shmuel Odes, Iris Dotan, Eric Ian Benchimol, Dan Turner

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Mira Stulman, Gili Focht, Dan Turner, The Juliet Keiden Institute of Pediatric Gastroenterology and Nutrition, Shaare Zedek Medical Center, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 9103102, Israel

Mira Stulman, Ora Paltiel, Braun School of Public Health and Community Medicine, Hadassah Medical Organization, Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem 9574869, Israel

Yiska Loewenberg Weisband, Clalit Research Institute, Tel Aviv 6209804, Israel

Shira Greenfeld, Amir Ben Tov, Maccabi Health Services and the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 6801296, Israel

Natan Ledderman, Meuhedet Health Services, Tel Aviv 6203854, Israel

Eran Matz, Leumit Health Services, Tel Aviv 6473704, Israel

Shmuel Odes, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel

Iris Dotan, Department of Gastroenterology, Rabin Medical Center, Petah Tikva 49100, Israel

Eric Ian Benchimol, Department of Paediatrics and Institute of Health Policy, Management and Evaluation, The Hospital for Sick Children, University of Toronto, Toronto M5G 1X8, ON, Canada

Eric Ian Benchimol, SickKids Inflammatory Bowel Disease Centre, Division of Gastroenterology, Hepatology and Nutrition, Child Health Evaluative Sciences, The Hospital for Sick Children and the SickKids Research Institute, Toronto M5G 1X8, Canada

Eric Ian Benchimol, ICES, Toronto M4N 3M5, Canada

Corresponding author: Dan Turner, MD, PhD, The Juliet Keiden Institute of Pediatric Gastroenterology and Nutrition, Shaare Zedek Medical Center, Faculty of Medicine, The Hebrew University of Jerusalem, Shmuel (Hans) Beyth St 12, Jerusalem 9103102, Israel. turnerd@szmc.org.il

Abstract

BACKGROUND

Israel has a high rate of Jewish immigration and a high prevalence of inflammatory bowel disease (IBD).

AIM

To compare IBD prevalence in first-generation immigrants *vs* Israel-born Jews.

METHODS

Patients with a diagnosis of IBD as of June 2020 were included from the validated epi-IIRN (Israeli IBD Research Nucleus) cohort that includes 98% of the Israeli population. We stratified the immigration cohort by IBD risk according to country of origin, time period of immigration, and age group as of June 2020.

RESULTS

A total of 33544 patients were ascertained, of whom 18524 (55%) had Crohn's disease (CD) and 15020 (45%) had ulcerative colitis (UC); 28394 (85%) were Israel-born and 5150 (15%) were immigrants. UC was more prevalent in immigrants (2717; 53%) than in non-immigrants (12303, 43%, $P < 0.001$), especially in the < 1990 immigration period. After adjusting for age, longer duration in Israel was associated with a higher point prevalence rate in June 2020 (high-risk origin: Immigration < 1990: 645.9/100000, ≥ 1990: 613.2/100000, $P = 0.043$; intermediate/low-risk origin: < 1990: 540.5/100000, ≥ 1990: 192.0/100000, $P < 0.001$). The prevalence was higher in patients immigrating from countries with high risk for IBD (561.4/100000) than those originating from intermediate-/low-risk countries (514.3/100000; $P < 0.001$); non-immigrant prevalence was 528.9/100000.

CONCLUSION

Lending support to the environmental effect on IBD etiology, we found that among immigrants to Israel, the prevalence of IBD increased with longer time since immigration, and was related to the risk of IBD in the country of origin. The UC rate was higher than that of CD only in those immigrating in earlier time periods.

Key Words: Epidemiology; Inflammatory bowel disease; Immigration; Environment

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Core Tip: In this nationwide study, we compared inflammatory bowel disease (IBD) rates between first-generation immigrants originating from countries of varying IBD risk *vs* Israel-born residents. Our focus on the Jewish population was aimed at narrowing the genetic variation of IBD that is usually present in immigration cohorts. We found that the prevalence rate was lower among patients from intermediate- and low-risk regions compared to patients from high-risk regions but in both, the prevalence increased in association with duration in Israel after immigration. This finding, especially among immigrants from intermediate- and low-risk countries, lends support toward the role of environmental factors in IBD pathogenesis in Israel.

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INTRODUCTION

The incidence of inflammatory bowel disease (IBD) is rising sharply in low socio-demographic index regions such as Africa, Asia, and South America[1-3]. Increasing rates have also been observed among immigrants from low-resource countries to developed countries[4-6], and to a lesser extent, also *vice versa* (*e.g.*, from the Faroe Islands to Denmark, where the excess ulcerative colitis (UC) risk in Faroese immigrants was no longer seen after immigration to the new country)[7], implying probable environmental triggers for developing IBD. However, these nationwide studies have included immigrants from various ethnicities[5,8,9] with different genetic backgrounds compared to the host country, hampering attention to the environmental effect on IBD risk. In contrast, in Israel, which is defined as a high prevalence country for IBD, reaching 0.59% of the total population in 2021, and 0.67% among the Jewish population[10,11], the vast majority of immigrants are Jewish or first-degree relatives of Jewish residents who share a similar genetic background[12]. This poses a unique opportunity to study the impact of environmental factors in a high-risk westernized country among immigrants and Israel-born residents with relatively similar genetic predisposition to IBD[13,14], originating from countries with varying degrees of IBD risk.

Israel is a melting pot of various ethnicities and cultures, characterized by waves of immigration from all over the world. As a country with a population of 9.2 million (as of June 2020), Israel has absorbed 1.5 million Jewish immigrants since 1990 with little population drift[15]. Israel has a universal healthcare system where all residents are insured by one of four Health Maintenance Organizations (HMOs), making administrative studies relatively accurate. However, previous Israeli studies comparing IBD rates among Israel-born residents *vs* immigrants were conducted in small regional cohorts[16-23].

We aimed to compare the rate of IBD in first-generation immigrants *vs* Israel-born residents using a nationwide cohort of patients with IBD. We also aimed to determine whether the duration of residence in Israel affects the rate of IBD in these immigrants. Finally, we aimed to examine whether the rate of IBD in immigrants is related to the IBD risk in their country of origin.

MATERIALS AND METHODS

We utilized the Israeli IBD Research Nucleus (epi-IIRN), a validated nationwide IBD cohort including 98% of the patients with IBD in Israel [50503 patients with IBD as of June 2020: 27490 with Crohn's disease (CD) and 23013 with UC]. Case-ascertainment algorithms were validated for each of the four HMOs in Israel [89% sensitivity, 99% specificity, 92% positive predictive value (PPV), 99% negative predictive value (NPV)]. Further algorithms classified disease type as CD or UC (92% sensitivity, 97% specificity, 97% PPV, 92% NPV)[10,24].

We constructed a prevalence cohort including all Jewish patients with IBD in the epi-IIRN database who had an active diagnosis by June 2020, stratified by Israel-born and first-generation immigrants. Our focus on the Jewish population was based on the Israeli Law of Return, which almost exclusively gives Jews the right to receive citizenship in Israel. Indeed, 99.4% of immigrants in the epi-IIRN cohort immigrated under this definition.

Country of birth and date of immigration were retrieved from the HMOs' electronic medical records for all patients with IBD. We categorized the countries of origin by degree of risk for IBD based on published systematic reviews[3,25] and according to Agrawal *et al*[5] as: Low risk [*i.e.,* incidence of CD, < 1.95/100000 person-years (PY) and UC, < 3.10/100,000 PY], intermediate risk (*i.e.,* CD, 1.95-6.38/100000 PY or UC, 3.10-7.71/100000 PY), and high risk (*i.e.,* CD, > 6.38/100000 PY or UC, > 7.71/100000 PY). Each risk category was further stratified into age groups: 0-34, 35-65, and 65+ years as of June 2020. Duration of residence in Israel was inferred by immigration time periods, divided as the years < 1990, 1990-2001, and 2002-2020, based on immigration reports from the Israeli Central Bureau of Statistics[12]. When further stratifying by risk in country of origin and age groups, time periods were divided as the years < 1990 and 1990 onwards (*i.e.,* at least 30 years of residence in Israel, or less than 30 years), again based on the national data published by the Israel Central Bureau of Statistics, with these time periods available for each risk stratum[15].

In order to minimize the possibility that IBD was diagnosed prior to immigration and was only new to the Israeli medical system, we performed a sensitivity analysis, which only included immigrants with at least a five-year lookback period from the date of immigration to the date of the first IBD code, as previously validated in the epi-IIRN cohort[24].

Patients who originated from countries with no risk data available or with missing data such as date of birth, country of origin, or immigration date were excluded. The date of death was obtained by linking each patient's unique identifying code by deterministic approach to the Israeli Ministry of Health national mortality database. Socioeconomic status scores were based on a scale of 1-10 and grouped into three categories: 1-3, low; 4-6, intermediate; and 7-10, high[26], and current residence in Israel was divided into rural and urban based on address categorization by the Israeli Central Bureau of Statistics.

Statistical analysis

Factors were compared between the cohorts using ANOVA and Kruskal-Wallis tests, corrected for multiple comparisons by the Bonferroni method, as well as chi-square tests where appropriate. Point prevalence rates were calculated by dividing the annual number of living patients by the average population as of 2020, reported per 100000 with 95% confidence intervals (CI) and age-standardized by the direct method to the Israeli population for comparison between the groups. Proportions were compared *via* chi-squared contingency tables. SPSS version 28 (IBM Corporation, Armonk, NY, United States) and R for statistical computing[27] were used for statistical analyses. The Institutional Review Board of Shaare Zedek Medical Center approved the study.

RESULTS

A total of 33544 Jewish patients were included in the study, of whom 18524 (55%) had CD and 15020 (45%) had UC; 85% were Israel-born (n = 28394) and 15% (n = 5150) were immigrants. Of the latter, 25 % (n = 1293) originated from high-risk countries, 70% (n = 3615) from intermediate-risk countries, and 5% (n = 242) from low-risk countries. Due to the small sample size in the low-risk group, immigrants from low- and intermediate-risk countries were grouped together and compared with those from high-risk countries. Data regarding the number of immigrants and the risk category assigned to each country are detailed in Supplementary Table 1. Immigrants from Poland (n = 191) and Switzerland (n = 12) were excluded from this analysis, because the last available IBD data were from 1951-1960 and 1960-1969, respectively. The majority of immigrants were from former USSR (Union of Soviet Socialist Republics) countries (56%) and Europe (21%), followed by North America (11%), the Middle East (6%), South America (4.4%), Africa (1.5%), and Asia (0.1%). Additional

Table 1 Basic characteristics of the inflammatory bowel disease cohort by June 2020 [*n* (%), mean ± SD and median (interquartile range) are displayed as appropriate][1]

	Origin from high-risk countries (*n* = 1293)	Origin from intermediate- and low-risk countries (*n* = 3857)	Israel-born (*n* = 28394)
Prevalence-crude rate[2]	561.4	514.3	528.9
Sex (Female)	651 (50)	1953 (51)	14234 (50)
Age at immigration	25 ± 18	25 ± 17	N/A
IBD phenotype (Crohn's disease)	685 (53)	1748 (45)	16091 (57)
Age at diagnosis (yr)	41 ± 18	46 ± 17	32 ± 15
Age at June 2020	43 ± 17	60 ± 17	54 ± 19
Immigration periods			
< 1990	501 (39)	1358 (35)	N/A
1990-2001	229 (19)	2199 (57)	
2002-2020	543 (42)	300 (8)	
Socioeconomic status			
Low	65 (5)	247 (7)	1348 (5)
Intermediate	550 (52.5)	2247 (58)	10680 (37)
High	646 (50)	1247 (32)	15589 (55)
Missing	32 (2.5)	116 (3)	777 (3)
Residential location			
Rural	111 (9)	155 (4)	3173 (11)
Urban	1182 (91)	3701 (96)	25221 (89)

[1]Proportions between groups were significantly different, $P < 0.001$.
[2]Per 100000.
IBD: Inflammatory bowel disease; N/A: Not applicable.

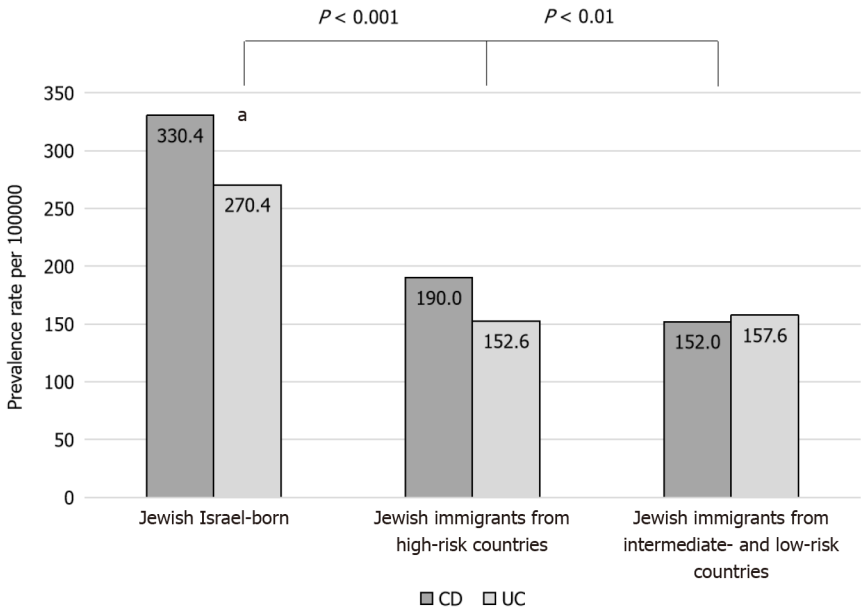
socio-demographic characteristics are provided in Table 1.

Among the Israel-born cohort, 16091 (57%) had CD and 12303 (43%) had UC, while in the immigration cohort, UC dominated: 2433 (47%) CD *vs* 2717 UC (53%) (odds ratio (OR) = 1.46 (95%CI: 1.38-1.55); $P < 0.001$]. UC dominated among immigrants from low- and intermediate-risk countries [1748 (45%) CD *vs* 2109 (55%) UC, $P < 0.001$], while immigrants from high-risk countries were more likely to be diagnosed with CD [685 (53%) *vs* 608 (47%), $P = 0.0023$)].

Crude IBD point prevalence rates in 2020 were highest among immigrants originating from high-risk countries [561.4 per 100000 (95%CI: 546.8-576.2)] followed by Israel-born residents [528.9 per 100000 (95%CI: 522.7-535.0)] and were lowest among immigrants from intermediate- and low-risk countries [514.3 per 100000 (95%CI: 493.2-535.9); $P < 0.001$]. In the immigration cohort, age-standardized point prevalence rates in 2020 were 342.6 per 100000 for immigrants originating from high-risk countries and 309.5 per 100000 for intermediate- and low-risk countries [OR = 1.1 (95%CI: 1.03-1.17); $P = 0.0041$]. For the Israel-born cohort, the standardized rate was twice as high (600.8 per 100000) as the entire immigration cohort (318.1 per 100000) [OR = 1.9 (1.65-2.17); $P < 0.001$]. When stratified by phenotype, CD prevalence rates were highest in the Israel-born cohort, followed by immigrants from high-risk countries, and were lowest among immigrants from intermediate- and low-risk countries (330.4 *vs* 190.0 *vs* 152.0 per 100000, respectively; $P < 0.001$; Figure 1).

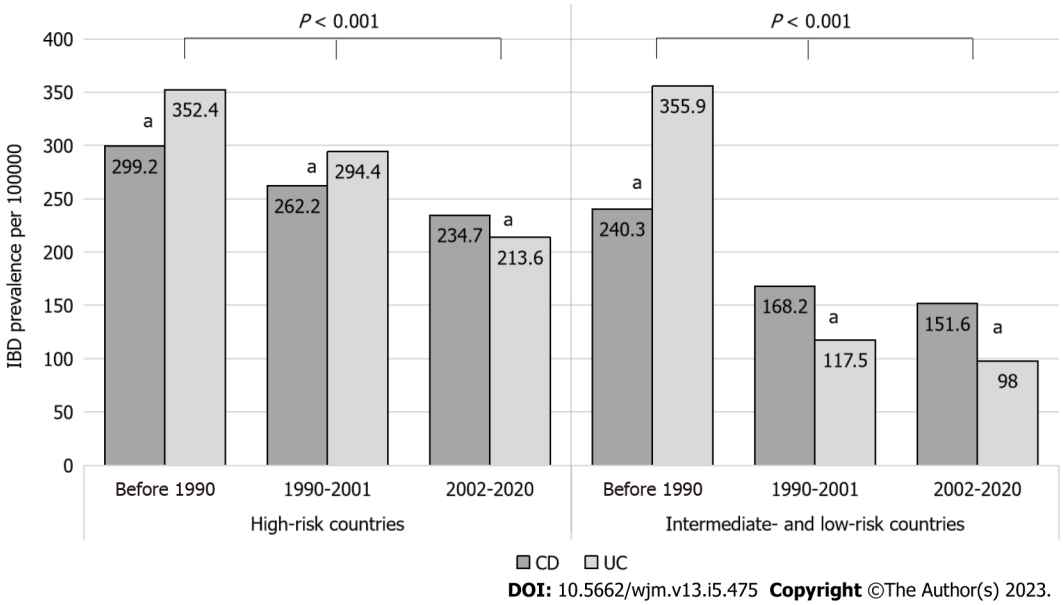
Among the immigrants, there was a significant association between longer duration of living in Israel and higher IBD prevalence rates, regardless of risk in the country of origin (Figure 2). CD became dominant among patients who immigrated after 1990 from intermediate- and low-risk countries, and from 2002 among patients who immigrated from high-risk countries. When stratifying by both IBD risk and age groups, prevalence for both CD and UC increased between time periods in each sub-group, with the exception of the group aged 35-64 years among immigrants from high-risk countries (Figure 3).

In the sensitivity analysis, there was a significant and positive association between longer duration in Israel and higher IBD prevalence rates for all countries of origin and among all age groups, including those aged 35-64 years (Supplementary Figure 1). For this analysis, 1057/5150 (21%) were excluded due to insufficient look-back period from the time of immigration; 1930 (47%) had CD and 2163 (53%) had UC; and 1115 (27%) originated from high-risk countries and 2978 (73%) from intermediate- and low-risk countries. UC was more common among individuals originating from intermediate- and low-risk countries [1571 (53%) UC *vs* 1407 (47%) CD, $P < 0.001$], as well as from high-risk countries [572



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Figure 1 Inflammatory bowel disease prevalence among Jewish Israel-born and first-generation immigrants in June 2020. [a]*P* < 0.01. CD: Crohn's disease; UC: Ulcerative colitis.



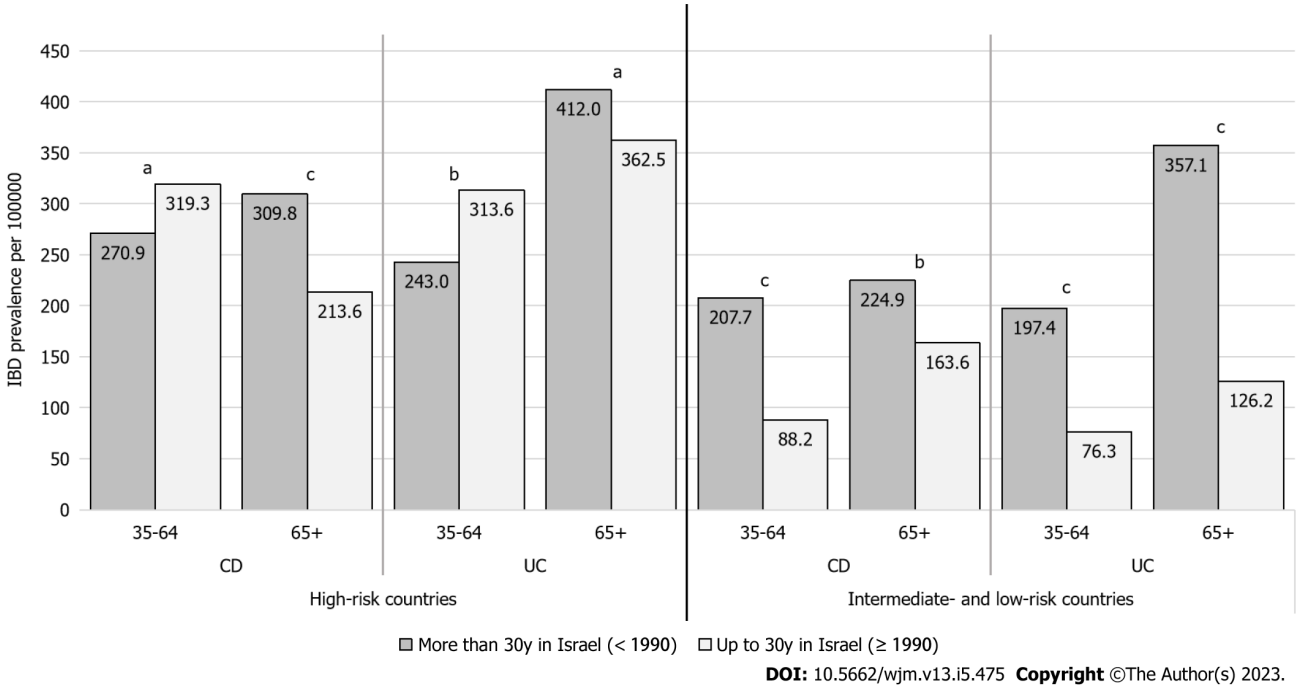
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Figure 2 Inflammatory bowel disease prevalence among Jewish immigrants originating from varying risk countries in June 2020, stratified by duration of residence in Israel. [a]*P* < 0.01. CD: Crohn's disease; UC: Ulcerative colitis.

(51%) UC *vs* 543 (49%) CD], although in the latter, the difference was not statistically significant ($P = 0.34$).

DISCUSSION

In this nationwide study, we focused on the Jewish population to narrow the genetic variation of IBD that is usually present in immigration cohorts. We found that the IBD prevalence rate was lower among patients originating from intermediate- and low-risk regions compared to patients from high-risk regions but in both, the prevalence increased in association with duration of living in Israel after immigration. This finding, especially among immigrants from intermediate- and low-risk countries, lends support toward the role of environmental factors in IBD pathogenesis in Israel. In accordance with our findings, nationwide studies in Canada[8,28] and Sweden[9] have reported a lower risk of IBD among immigrants from low-risk countries compared to the non-immigrant population.



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Figure 3 Inflammatory bowel disease prevalence among Jewish immigrants from varying risk countries in June 2020, stratified by age group and by duration of residence in Israel. [a]*P* < 0.05; [b]*P* < 0.01; [c]*P* < 0.001. CD: Crohn's disease; UC: Ulcerative colitis.

Previous studies on immigration to Israel were small and regional such as from the Beer Sheva region (1961-1985 and 1979-1987)[16,17], Central Israel (1970-1980)[18,19], the Kinneret sub-district of Northern Israel (1965-1989)[20,21], and Israeli Kibbutz settlements (1987-2007)[22,23]. These studies mainly compared IBD prevalence and incidence between Israel-born *vs* European-American-born and Asian-African-born Jewish immigrants. Most of these studies showed higher prevalence rates in European-American-born immigrants or Israel-born individuals, similar to our study. Our analysis, however, included all immigrants in Israel, including a substantial number of patients from low- and intermediate-risk countries, such as former USSR countries, who initially demonstrated higher UC rates but shifted to the local higher rate of CD in association with longer duration in Israel.

Although UC was predominant among patients who immigrated before 1990, CD became more prominent during the period from 2002 onwards, around the time when the switch from UC to CD dominance occurred in Israel (in 2006, as demonstrated in our previous study)[10]. High UC rates have been previously demonstrated in immigrants from both low-risk[29,30] and high-risk countries[7]. UC dominance, at least at first, may be explained by the hypothesis that UC is predominantly driven by exposure to environmental risk factors that may occur at any point in life, whereas CD is predominantly driven by genetic-environmental interactions in early life at critical stages of immunologic development [31].

Environmental risk factors affecting immigrant populations worldwide may include changes in diet, water supply, hygiene, socioeconomic status, access to the health care system, use of antibiotics, urbanization, and possibly psychological stress[32-34]. Vangay *et al*[35] demonstrated 'westernization' of the gut microbiome among 281 Southeast Asian individuals who immigrated to the United States. That study demonstrated loss of native gut microbiome diversity and function, amplified by longer duration in the United States.

Our study had several strengths. This study was the first to describe and compare the immigrant *vs* Israel-born IBD population in Israel on a national scale, who likely have similar genetic backgrounds, highlighting the role of environmental factors in IBD development in Israel. We utilized the national epi-IIRN cohort, which is based on validated algorithms accurately distinguishing patients with IBD, IBD type, and incident cases[24], thus minimizing selection bias and loss to follow-up. In Israel, healthcare coverage is universal, minimizing healthcare access bias between the immigrant and Israel-born groups. We conducted a sensitivity analysis that excluded patients without sufficient look-back period (and who may have had the disease at immigration), in an effort to minimize lead-time bias.

Limitations of our study include lack of access to national data necessary to calculate person-time and incidence rate ratios, as well as lack of data regarding lifestyle habits such as smoking and dietary intake among the immigration cohorts in the origin countries, as well as in Israel. Thus, we could not quantify the impact of specific environmental risk factors. We also excluded immigrants from countries without known IBD risk data, although the IBD immigration from these cohorts was negligible, such as some of the African countries (Supplementary Table 1).

It is almost impossible to fully disentangle periods of immigration and cohort effects explored in immigration studies in a meaningful way[36,37]. In our study, it is possible that there are periods of arrival cohort effects, but these cannot be distinguished from duration of residence effects. However, we did stratify the analysis by IBD risk in the country of origin, which may account for the cohort effect to a certain extent.

CONCLUSION

In conclusion, we found a high prevalence rate of IBD among immigrants to Israel and identified a positive association between duration of time after immigration and IBD prevalence, alluding to environmental risk factors in Israel. Future studies should explore associations between immigration with time to IBD onset, and should examine specific environmental factors among immigrants to further our understanding of the elusive IBD etiology.

ARTICLE HIGHLIGHTS

Research background

Israel has a high rate of Jewish immigration and a high prevalence of inflammatory bowel disease (IBD). The study of IBD among immigrants is of paramount importance for several compelling reasons. Immigration itself facilitates population growth and changes in demographics, thereby influencing prevalence trends. Moreover, immigration introduces individuals to new environments, dietary habits, hygiene practices, and lifestyle behaviors, which can significantly alter their risk of developing IBD as they assimilate into their host countries.

Research motivation

Investigating IBD among immigrants provides a unique opportunity to dissect the complex interplay between genetics, environment, and migration in disease development, especially if focusing on a specific ethnic group of immigrants with similar predisposition to IBD. In this study, we compared IBD rates between first-generation immigrants originating from countries of varying IBD risk *vs* Israel-born residents, focusing specifically on the Jewish population in an effort to narrow the genetic variation of IBD that is usually present in immigration cohorts, in an increasingly interconnected world.

Research objectives

We aimed to compare the rate of IBD in first-generation immigrants *vs* Israel-born residents using a nationwide cohort of patients with IBD. We also aimed to determine whether the duration of residence in Israel affects the rate of IBD in these immigrants. Finally, we aimed to examine whether the rate of IBD in immigrants is related to the IBD risk in their country of origin.

Research methods

Patients with a diagnosis of IBD as of June 2020 were included from the validated Israeli IBD Research Nucleus cohort that includes 98% of the Israeli population. We stratified the immigration cohort by IBD risk according to country of origin, time period of immigration, and age group as of June 2020.

Research results

Of the 33544 Jewish patients that were ascertained, 18524 (55%) had Crohn's disease and 15020 (45%) had ulcerative colitis (UC); 28394 (85%) were Israel-born and 5150 (15%) were immigrants. UC was more prevalent in immigrants (2717; 53%) than non-immigrants (12303, 43%, $P < 0.001$), especially in the < 1990 immigration period. The prevalence was higher in patients immigrating from countries with high risk for IBD (561.4/100000) than those originating from intermediate-/low-risk countries (514.3/100000; $P < 0.001$); non-immigrant prevalence was 528.9/100000. After adjusting for age, longer duration in Israel was associated with a higher point prevalence rate in June 2020 (high-risk origin: Immigration < 1990: 645.9/100000, ≥ 1990: 613.2/100000, $P = 0.043$; intermediate/low-risk origin: < 1990: 540.5/100000, ≥ 1990: 192.0/100000, $P < 0.001$).

Research conclusions

Our focus on the Jewish population was aimed at narrowing the genetic variation of IBD that is usually present in immigration cohorts. We found that the prevalence rate was lower among patients from intermediate-and low-risk regions compared to patients from high-risk regions but in both, the prevalence increased in association with duration in Israel after immigration. This finding, especially among immigrants from intermediate- and low-risk countries, lends support toward the role of environmental factors in IBD pathogenesis in Israel.

Research perspectives

Future studies should explore associations between immigration with time to IBD onset, and should examine specific environmental factors among immigrants to further our understanding of the elusive IBD etiology.

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FOOTNOTES

Author contributions: Stulman M designed and conceptualized the study, collected, analyzed, and interpreted the data, and drafted the manuscript; Focht G, Loewenberg Weisband Y, Greenfeld S, Ben Tov A, Ledderman N, and Matz E contributed to data acquisition; Paltiel O, Odes S, Dotan I, and Benchimol EI contributed in data analysis and interpretation; Turner D designed and conceptualized the study, contributed to data analysis and interpretation, and drafted the manuscript.

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Country/Territory of origin: Israel

ORCID number: Mira Stulman 0000-0003-2934-7393; Shmuel Odes 0000-0002-8452-9926; Iris Dotan 0000-0002-8005-7706; Eric Ian Benchimol 0000-0001-8855-3598.

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Basic Study

Sequential extraction of RNA, DNA and protein from cultured cells of the same group

Ying-Yu Cui

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Ying-Yu Cui, Department of Cell Biology, Institute of Medical Genetics, Key Laboratory of Arrhythmias of the Ministry of Education of China, Tongji University School of Medicine, Shanghai 200331, China

Corresponding author: Ying-Yu Cui, PhD, Associate Professor, Teacher, Department of Cell Biology, Institute of Medical Genetics, Key Laboratory of Arrhythmias of the Ministry of Education of China, Tongji University School of Medicine, No. 500, Zhennan Road Putuo District, Shanghai 200331, China. yycui@tongji.edu.cn

Abstract

BACKGROUND

Efficient extraction of nucleic acids and proteins (ENAP) from cells is a prerequisite for precise annotation of gene function, and has become laboratory routine for revealing the mysteries of life. However, cell samples are often from different culture dishes, resulting in inevitable experimental errors and sometimes poor repeatability.

AIM

To explore a method to improve the efficiency of ENAP, minimizing errors in ENAP processes, enhancing the reliability and repeatability of subsequent experimental results.

METHODS

A protocol for the sequential isolation of RNA, DNA, and proteins from the same cultured HepG2 cells using RNAzol reagent is presented here. The first step involves culturing HepG2 cells to the exponential phase, followed by the sequential isolation of RNA, DNA, and proteins from the same cultured cells in the second step. The yield of nucleic acids and proteins is detected in the third step, and their purity and integrity are verified in the last step.

RESULTS

The procedure takes as few as 3-4 d from the start to quality verification and is highly efficient. In contrast to the existing kits and reagents, which are primarily based on independent isolation, this RNAzol reagent-based method is characterized by the sequential isolation of RNA, DNA, and proteins from the same cells, and therefore saves time, and has low cost and high efficiency.

CONCLUSION

The RNA, DNA, and proteins isolated using this method can be used for reverse transcription-polymerase chain reaction, polymerase chain reaction, and western blotting, respectively.

Key Words: Sequential extraction; Ribonucleic acid; Deoxyribonucleic acid; Protein; Cultured cells

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Core Tip: Sequential extraction of nucleic acids and proteins from cultured cells of the same group. Life is a way of material (mainly protein and nucleic acid) movement, and health lies in movement. Cell is the most fundamental structural and functional unit of life. Therefore, the effective isolation of nucleic acids and proteins from cells is the foundation and prerequisite for revealing the mysteries of life. However, during laboratory routine for isolation of nucleic acids and proteins, cell samples are often from different culture dishes, usually leading to inevitable experimental errors and sometimes poor repeatability. The present research tries to explore the possibility to simultaneously isolate nucleic acids and proteins from the same sample, while reducing experimental errors and ensuring consistency during experimentation. The present study established a selective protocol for sequential isolation of RNA, DNA and proteins from the same cells with the characteristics of easy operation, rapid extraction and high efficiency. RNAzol reagent was used for the sequential isolation of RNA, DNA, and proteins from the same cultured HepG2 cells, resulting in a novel protocol containing four steps. A protocol for sequential isolation of RNA, DNA and proteins was established and the procedure takes as few as 3-4 d from the start to quality verification and is highly efficient. The quality of RNA, DNA and proteins isolated through sequential isolation protocol can be used for reverse transcription - polymerase chain reaction (PCR), PCR and western blot, respectively. The present procedure is not only easy, rapid and high efficient, but also economical and practical, especially for researchers in developing and underdeveloped countries.

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INTRODUCTION

The essence of life relies on molecular kinetics, which primarily involves the interactions of nucleic acids (DNA and RNA) and proteins. Therefore, the extraction of nucleic acids and proteins is crucial for deciphering the secrets of life. It is becoming increasingly important for researchers in the post-genomic era to efficiently isolate nucleic acids and proteins of high purity and integrity from cultured cells. Various commercial kits are available for the isolation of nucleic acids and proteins, using various methods[1]. Recently, methods for RNA[2], DNA[3] and protein[4,5] micro-isolation have been updated, respectively, and simultaneous isolation of RNA and DNA or protein has also been reported[6,7], but there has been no reported method for simultaneous isolation of RNA, DNA, and protein from cultured cells of the same group. Methods for the independent extraction of nucleic acids and proteins from different groups of cultured cells comprise several steps, are expensive, and time-consuming; these factors affect the productivity, purity, and integrity of the isolated samples. Most importantly, it is very difficult to ensure that the number of cells, cell growth status, and metabolic status of the cultured cells are consistent across the different groups, which inevitably increases the chances of experimental errors between earlier and later experiments. Although the complete elimination of errors is not achievable during experimentation, it is essential to minimize errors as far as possible. The quality of the RNA and proteins isolated from different groups of cultured cells could be a key factor responsible for the inconsistencies in gene expression data obtained by reverse transcription (RT)-polymerase chain reaction (PCR) and western blotting that are often observed. By referring to related literature[8-10] and repeated experimentation, the present study established a relatively rapid procedure for the sequential extraction of RNA, DNA, and proteins from the same group of cultured cells. The method described herein is not only easy and inexpensive, but also has high reproducibility, comparability, and credibility, and ensures consistency during experimentation.

MATERIALS AND METHODS

Reagents

The following reagents were used in this study for the successive extraction of nucleic acids and proteins: RPMI 1640 (HyClone), penicillin-streptomycin (10000 units/mL penicillin and 10000 μg/mL streptomycin; Invitrogen), HEPES (Sigma, United States), fetal bovine serum (FBS; Sijiqing, China), RNAzol (Cohen-Bio Corp., Beijing, China, lot no. NA6111), sodium dodecyl sulfate (SDS; Amresco), agarose (Fluka, Spain), diethyl pyrocarbonate (DEPC; Sigma), acrylamide (BBI, Canada), bis-acrylamide (BBI), tetramethylethylenediamine (TEMED; Sigma), ammonium persulfate

(Sigma,), guanidine hydrochloride (BBI, Canada), Tris (Sigma), glycine (Sigma), ethidium bromide (E.B; Sigma), MOPS (Serva, Sino-American Biotechnology Co.), PRO-STAIN™ protein marker II (SBS Genetech Co., Beijing, China), horseradish peroxidase-anti-glyceraldehyde-3-phosphate dehydrogenase (HRP-anti-GAPDH; Kangchen Bio-tech, Shanghai, China), and sediment type mono-ingredient TMB substrate solution (PA108-01, Tiangen Biotech Co., Beijing, China).

Primers

The following primers were used in this study: c-Myc: Forward: 5′-AGCAAACCTCCTCACAGC-3′, and reverse: 3′-GATGCCTTGAGAACACGC-5′ (GenBank accession number: NM_002467).

Equipment

The following equipment was used for the sequential extraction of nucleic acids and proteins: CO_2 water jacketed incubator (Thermo Forma MODEL 3111, series II, HEPA FILTER, Forma Scientific Inc.), inversion microscope (XDS-1B, COIC), an agarose gel electrophoresis apparatus (DYY-III-4, LiuYi Corp. Beijing), SDS-polyacrylamide gel electrophoresis (PAGE) apparatus (POWER-PAC200/300, Bio-Rad), a MultiMage™ Light Cabinet (Alpha Innotech Corporation), and a ultraviolet spectrometer (Ultrospec®2100 Pro, Amersham Pharmacia Biotech).

Cell culture

Duration: 1-3 d, depending on the cell line used.

The cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 10 mmol/L HEPES at pH 7.4, and maintained at 37 °C in a humidified incubator with 5% CO_2. When the cells reached a confluence of 75%, they were detached with 0.25% trypsin and subsequently seeded into 24-well microtiter plates at a density of 10^4 cells /mL with 2 mL complete culture media /well, and cultured in a 5% CO_2 incubator for 24, 48, 72, and 96 h. The cells were collected from three wells at each time point and counted with a hemocytometer. The mean number of cells calculated from the three wells represented the y-axis value, and the time point was represented on the x-axis of a cell growth curve, which was analyzed to identify the exponential growth phase of the cells.

RNA isolation

Duration: Approximately 1 h.

Homogenization: The cells were directly lysed in the exponential phase of growth in a culture dish by adding 1 mL RNAzol reagent to a dish of diameter 3.5 cm, and the cell lysate was pipetted several times for homogenization.

The homogenized samples were incubated for 5 min at room temperature (RT, 25 °C) to allow the complete dissociation of nucleoprotein complexes.

The samples were centrifuged at 11000 × *g* for 10 min at 4 °C, following which the clear supernatant solution was transferred to a fresh 1.5 mL Eppendorf tube.

A 0.2 mL aliquot of chloroform was added to the Eppendorf tube and the sample tubes were securely capped. The tubes were vigorously shaken by hand for 15 s and incubated at RT for 3 min.

The samples were centrifuged at 11000 × *g* for 15 min at 4 °C. Following centrifugation, the mixture separated into a lower, phenol-chloroform phase, an interphase, and an upper colorless aqueous phase. The RNA remained exclusively in the aqueous phase, the DNA remained in the interphase, and the proteins were retained in the lower organic phase. The volume of the aqueous phase was approximately 60% of the volume of RNAzol reagent used for homogenization. The aqueous, interphase, and organic phases were collected in fresh 1.5 mL Eppendorf tubes, and the tubes containing the interphase and organic phase were stored at 4 °C for the isolation of DNA and proteins.

A 0.5 mL aliquot of isopropyl alcohol was added to the tube containing the aqueous phase, mixed evenly, and allowed to stand at room temperature for 10 min.

The mixture was centrifuged at 11000 × *g* for 10 min at 4 °C and the supernatant was discarded. The RNA precipitate, often invisible before centrifugation, formed a gel-like pellet on the sides and bottom of the tube.

At least 1 mL of 75% ethanol was added for washing the RNA pellet, once.

The sample was mixed by vortexing and subsequently centrifuged at a speed < 7500 × *g* for 5 min at 4 °C. The supernatant obtained after centrifugation was discarded.

The RNA pellet was air-dried for 5-10 min. The RNA was dissolved in an appropriate volume of RNase-free water by pipetting the solution a few times and incubating for 10 min at 55-60 °C. A 2 μL aliquot of the RNA solution was diluted 200-fold for detection of absorbance (A) at 260 nm, and the remnant was stored at - 80 °C until further use.

DNA isolation

Duration: Approximately 1 h.

Any remaining aqueous phase was removed from the interphase layer and discarded, and 0.3 mL of 100% ethanol was added per milliliter of RNAzol reagent used for the initial homogenization of the interphase and phenol phases. The samples were mixed by inversion and allowed to stand at room temperature for 3 min.

The DNA was sedimented by centrifugation at a speed less than 2000 × *g* for 5 min at 4 °C. The phenol-ethanol supernatant was then transferred to a fresh tube and stored at 4 °C for protein isolation.

A 0.3 mL aliquot of 0.1 M sodium citrate-10% ethanol solution was added to wash the DNA pellet, at least twice. During each wash, the DNA pellet was kept in the washing solution for 30 min at room temperature (with periodic



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Figure 1 Determination of the integrity of the total RNA isolated from the cultured HepG2 cells. Electrophoresis of the total RNA on a formaldehyde-denaturing agarose gel and subsequent staining with 0.5 mg/mL ethidium bromide revealed discrete bands corresponding to 28S, 18S, and 5S RNA molecules.

mixing) and centrifuged at 2000 × *g* for 5 min at 4 °C.

The DNA pellet was suspended in 75% ethanol (1.5-2 mL of 75% ethanol per mL of RNAzol reagent), stored for 20 min at room temperature (with periodic mixing), and centrifuged at 2000 × *g* for 5 min at 4 °C.

The DNA was air-dried for 5-15 min in an open tube. The DNA isolated from 10^6 cells was dissolved by adding 20-60 µL of 8 mmol/L NaOH, such that the concentration of the isolated DNA reached 0.2-0.3 µg/µL.

Protein isolation

Duration: Approximately 1 h.

For protein isolation, 1.5 mL of isopropanol was added to the previously obtained phenol-ethanol supernatant (approximate volume: 0.8-1 mL of RNAzol reagent), and allowed to stand at room temperature for 10 min.

The protein precipitate was sedimented by centrifugation at 12000 × *g* for 10 min at 4 °C, and the supernatant was discarded.

A 2 mL solution of 0.3 M guanidine hydrochloride in 95% ethanol was added to wash the protein pellet, thrice. During each wash cycle, the protein pellet was kept in the wash solution for 20 min at room temperature (15-30 °C) and centrifuged at 7500 × *g* for 5 min at 4 °C. After the final wash, the protein pellet was vortexed in 2 mL ethanol, stored in ethanol for 20 min at room temperature, and finally centrifuged at 7500 × *g* for 5 min at 4 °C.

The protein pellet was air-dried for 5-10 min and dissolved in 50 µL of 1% SDS by pipetting. Notably, the complete dissolution of the protein pellet might require incubating the sample at 50 °C. Any insoluble material was sedimented by centrifugation at 10000 × *g* for 10 min at 4 °C, and the supernatant was transferred to a fresh tube. A 1 µL aliquot of the sample was subsequently used for detecting the concentration and purity of the isolated proteins, and 10 µL of the sample was used for western blotting. The remainder was stored at −20 °C for future use.

RESULTS

The expected yields of RNA, DNA, and proteins from 1 × 10^6 cultured HepG2 cells are 5-10 µg, 4-7 µg, and 10-12 µg, respectively. Nucleic acids and proteins have an $A_{280}/A_{260} \geq 1.8$ when diluted with Tris-EDTA buffer (TE; 10 mmol/L Tris, 1 mmol/L EDTA, pH 8.0) and 0.1% SDS, respectively.

Electrophoresis of the isolated RNA on a formaldehyde-denaturing agarose gel and subsequent staining with ethidium bromide revealed discrete bands of high molecular weight, corresponding to RNA molecules of size 7-15 kb (mRNAs and hnRNAs), two predominant ribosomal RNA bands of size ~5 kb and ~2 kb (28S and 18S rRNAs, respectively), and a low molecular weight RNA molecule of 0.1-0.3 kb (tRNA, 5S) (Figure 1).

The total RNA obtained using this method is free from contamination with protein and DNA and can be used for molecular cloning (RT-PCR) (Figure 2).

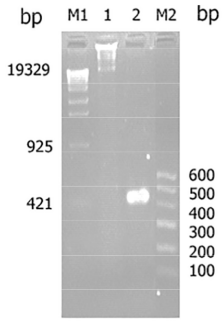
The DNA isolated using this method can be used for the detection of integrated foreign genetic materials by using PCR and restriction endonucleases.

The resulting protein isolated by this method can be analyzed for the presence of specific proteins by western blotting (Figure 3).

Notes

The cells should be lysed directly in a culture dish with appropriate RNAzol reagent only when they are in the exponential phase of growth, *i.e.* only cells in the exponential phase of growth should be used for isolation.

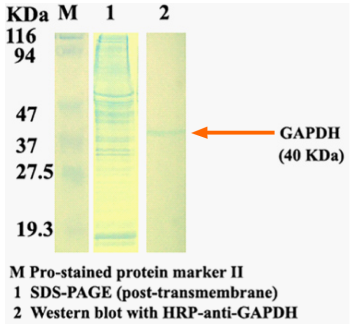
Disposable gloves should be worn always, and sterile, disposable plasticware and automatic pipettes should be reserved for RNA isolation. The glassware and plasticware should be kept RNase-free during RNA isolation to protect the RNA from contamination and degradation by RNases.



M1 λDNA/Eco 1301
1 Genomic DNA
2 c-Myc fragment
M2 DNA 100 bp marker

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Figure 2 Identification of genomic DNA and determination of the quality of the total RNA isolated from cultured HepG2 cells. Electrophoresis of the genomic DNA and products of reverse transcription (RT)-polymerase chain reaction (PCR) amplification of the c-Myc fragment on a 2.0% agarose gel and subsequent staining with 0.5 mg/mL ethidium bromide revealed the presence of 1.0-20 kb genomic DNA fragments (Lane 1), and a 500 bp c-Myc fragment that was amplified by RT-PCR using the total RNA as the template (Lane 2).



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Figure 3 Identification of the quality of the total proteins isolated from the same cultured HepG2 cells. The total proteins were separated on 12% gels by SDS-PAGE (Lane 1). Following transfer onto a polyvinylidene fluoride membrane, the 40-kDa GAPDH-specific band was blotted with HRP-anti-GAPDH and observed using sediment type mono-ingredient TMB substrate solution (Lane 2).

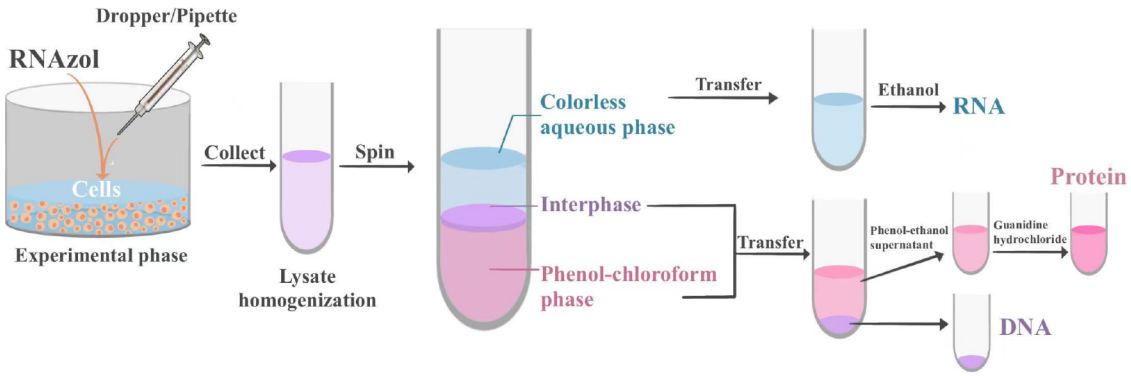
The isolated RNA and DNA samples should not be dried by centrifugation under vacuum.

DISCUSSION

Understanding the functions of genes in the postgenomic era is crucial for deciphering cellular mechanisms. Therefore, the development of effective methods of cell culture and novel techniques for the isolation of RNA, DNA, and proteins from cultured cells, especially the microextraction of nucleic acids and proteins from < 10^6 cultured cells, have become increasingly important. The protocol described herein offers such a method for isolating nucleic acids and proteins with relative rapidity and efficiency. The results demonstrate that the procedure allows the sequential isolation of RNA, DNA, and protein with high purity and integrity. The advantage of this method is that it allows the almost synchronous isolation of nucleic acids and proteins from the same cultured cells, which not only saves time, money, manpower, and material resources, but also preserves the identity of the isolated materials and enhances the reproducibility and reliability of the experimental results.

RNAzol is a ready-to-use reagent that is used for the isolation of total RNA from cells. The reagent consists of a monophasic solution of phenol and guanidine isothiocyanate, and the RNAzol-based technique of RNA isolation is superior to the single-step RNA isolation method developed by Chomczynski and Sacchi[8,9]. RNAzol maintains the integrity of the RNA during cell lysis and dissolution of cellular components. The addition of chloroform followed by centrifugation separates the solution into an aqueous phase and an organic phase, and the RNA remains exclusively in the aqueous phase. The RNA is recovered by precipitating the aqueous phase with isopropyl alcohol. The DNA in the interphase layer can then be isolated by precipitation with ethanol, and the proteins can be isolated from the organic phase by an additional precipitation step with isopropyl alcohol[4,10].

In the classical isolation methods, RNA, DNA, and proteins are extracted independently, which requires the preparation of a triplet group of cells as well as several reagents that need to be added separately during isolation. These protocols involve numerous steps and are time-consuming. In these conditions, RNA can easily be contaminated and



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Figure 4 Flow chart of ENAP protocol.

degraded by extraneous RNases. Therefore, the operational difficulty of the classical isolation methods is higher and the chances of successful isolation are decreased. Additionally, the various commercially available kits for the extraction of nucleic acids are expensive. RNAzol reagent facilitates the sequential isolation of RNA, DNA, and proteins from the same cultured cells, and preserves the identity of the isolated materials. The method developed herein has several advantages, including good comparability and reproducibility, a simple protocol, short duration of experimentation, improved work efficiency, reduced chance of RNA degradation, and it does not require the use of proteinase inhibitors for isolating single proteins. The quality of the isolated RNA, DNA, and proteins was validated by formaldehyde-denaturing agarose gel electrophoresis, RT-PCR, SDS-PAGE, and western blotting. And the findings reveal that the isolated nucleic acids and proteins can be used in molecular biology studies. Of note, this protocol is not suitable for lifelong cells without proliferative ability, *e.g.* neural cells and myocardial cells, or cells with relatively weak proliferative ability, *e.g.* stem cells with relatively small numbers.

CONCLUSION

Together, the present study describes a novel protocol for the sequential micro-extraction of RNA, DNA, and proteins from the same cells (Figure 4). The procedure has easy operation, allows rapid isolation, has high efficiency, and is economical and practical, especially for researchers in developing countries.

ARTICLE HIGHLIGHTS

Research background

Life is a way of material (mainly protein and nucleic acid) movement and health lies in movement. Cell is the most fundamental structural and functional unit of life, therefore, the effective isolation of nucleic acids and proteins from cells is the foundation and prerequisite for revealing the mysteries of life. However, during laboratory routine for isolation of nucleic acids and proteins, cell samples are often from different culture dishes, usually leading to inevitable experimental errors and sometimes poor repeatability.

Research motivation

The present research tries to explore the possibility to simultaneously isolate nucleic acids and proteins from the same sample, while reducing experimental errors and ensuring consistency during experimentation.

Research objectives

The present study established a selective protocol for sequential isolation of RNA, DNA and proteins from the same cells with the characteristics of easy operation, rapid extraction and high efficiency.

Research methods

RNAzol reagent was used for the sequential isolation of RNA, DNA, and proteins from the same cultured HepG2 cells, resulting in a novel protocol containing four steps.

Research results

A protocol for sequential isolation of RNA, DNA and proteins was established and the procedure takes as few as 3-4 d from the start to quality verification and is highly efficient.

Research conclusions

The quality of RNA, DNA and proteins isolated through sequential isolation protocol can be used for reverse transcription (RT) - polymerase chain reaction (PCR), PCR and western blot, respectively.

Research perspectives

The present procedure is not only easy, rapid and high efficient, but also economical and practical, especially for researchers in developing and underdeveloped countries.

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FOOTNOTES

Author contributions: Cui YY designed and performed the research, and analyzed the data and wrote the manuscript.

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ORCID number: Ying-Yu Cui 0000-0002-6324-0795.

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ORIGINAL ARTICLE

Basic Study

Urine exosome mRNA-based test for monitoring kidney allograft rejection: Effects of sample transportation and storage, and interference substances

Matt McFaul, Chris Ventura, Sean Evans, Halil Dundar, Marc J Rumpler, Christopher McCloskey, Dave Lowe, Alexandre V Vlassov

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Matt McFaul, Chris Ventura, Sean Evans, Halil Dundar, Marc J Rumpler, Christopher McCloskey, Dave Lowe, Alexandre V Vlassov, Department of Research and Development, Thermo Fisher Scientific, West Hills, CA 91304, United States

Corresponding author: Alexandre V Vlassov, PhD, Director, Department of Research and Development, Thermo Fisher Scientific, 22801 Roscoe Blvd, West Hills, CA 91304, United States. sasha.vlassov@thermofisher.com

Abstract

BACKGROUND

Exosomes are 30-150 nm nanovesicles with sophisticated nucleic acids cargo, actively secreted by all cells within human body, and found in abundance in all body fluids, including urine. These extracellular vesicles have tremendous potential for next generation diagnostics, theoretically enabling noninvasive assessment of organ and tissue function *via* liquid biopsy analysis.

AIM

Recently, feasibility of an exosomal molecular test was demonstrated for post-organ transplant monitoring: Analysis of urine-derived exosomal mRNA cargo allowed early detection of kidney allograft rejection. Here, we further studied urine-derived exosomes and their mRNA content as a highly promising diagnostic modality. This included stability studies of urine samples and exosomal mRNA upon transportation from the point of collection to a centralized testing facility, short-term storage of urine at different conditions upon receipt till the point molecular assay is performed, and effects of various potentially interfering substances on the downstream quantitative polymerase chain reaction (qPCR) assay.

METHODS

The urine specimens were stored at various conditions and pre-processed in different ways. Next, samples were passed through the columns to capture all extracellular vesicles, the vesicles were lysed to release their content and the exosomal RNA was purified on the mini-columns, reverse transcription was performed, next pre-amplification, followed by a qPCR analysis for a panel of

mRNA markers.

RESULTS

To ensure exosomal RNA integrity, the harvested urine specimens should be shipped refrigerated, by overnight delivery. Urine can next be stored at the test site for up to 1 wk at 4 °C, and long term should be frozen at -80 °C. Urine specimens must be centrifuge at low G-force to deplete cells and debris, to ensure consistent top results in downstream molecular assays. All commonly used medications (tacrolimus, cyclosporin A, mycophenolic acid, everolimus, sirolimus, ascomycin, teriflunomide) were tested and confirmed that they do not cause assay interference.

CONCLUSION

mRNA from urine-derived exosomes was shown to be stable across a broad range of conditions and produced accurate results when analyzed *via* qPCR assay for detection of kidney allograft rejection. We identified the most optimal conditions for every step of the process, ensuring pre-analytical sample integrity and robust qPCR results.

Key Words: Kidney allograft; Post-transplant monitoring; Liquid biopsy; Exosome; mRNA; Quantitative polymerase chain reaction

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Core Tip: Recently, it was demonstrated that analysis of urine-derived exosomal mRNA allows early detection of kidney allograft rejection. We further studied exosomes and their mRNA content as a highly promising diagnostic modality. This included stability studies of urine samples and exo-mRNA upon transportation from the point of collection to centralized testing facility, short-term storage of urine at different conditions upon receipt till the point molecular assay is performed, and effects of various interfering substances on the quantitative polymerase chain reaction assay. mRNA from the urine-derived exosomes was proven to be stable across broad range of conditions and produce robust results in post-transplant monitoring assays.

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INTRODUCTION

There is an urgent need for advanced post-transplant monitoring molecular tests, allowing to rapidly determine allograft rejection at the earliest stage using non-invasively collected patients' samples. At the moment one of the most widely utilized tests is serum creatinine, which has a number of limitations[1,2]. The next gen molecular assay, now rather widely used in laboratories world-wide is based on cell free DNA[3,4]. This test analyses percentage of the donor-derived (dd) cfDNA in the blood of organ recipient, and if the fraction increases over time and crosses a predetermined threshold (typically 1% for kidney transplants)- this indicates organ rejection. Although this test was already commercialized a few years ago, its specificity is suboptimal, as other-than-allograft rejection events can also trigger increase in dd cfDNA percentage. This test does not work well for detection of the early stage organ rejection (TCMR1a). Also, this approach is minimally invasive, rather than non-invasive- as it requires the collection of a patient's blood (which is an inconvenience for immunosuppressed individuals). Consequently, clinicians still need sensitive and specific biomarkers that overcome the above listed limitations of cfDNA-based tests for post-transplant monitoring.

Exosomes are recently discovered nanovesicles secreted by all cells within human body and found in abundance in all body fluids. They contain very sophisticated cargo of nucleic acids and proteins, reflecting the content of parental cells[5-9]. Exosomes and other extracellular vesicles have tremendous potential as biomarkers for disease diagnosis, as they theoretically should allow access to the health state of all organs and tissues *via* liquid biopsy analysis, using not only blood, but also urine, saliva, and other non-invasively collected samples[10-13]. The value of urinary exosomes in post-transplant diagnostics was demonstrated in a recent study for early detection of kidney transplant rejection[14]. Notably, urine collections are better suited for active surveillance monitoring compared to frequent blood draws and urinary exosomes exhibit promising clinical utility for detecting early kidney allograft rejection and stratifying rejection etiology.

In this study, we further characterized the aforementioned assay to understand the preanalytical limitations for an eventual migration into the clinical diagnostic domain. An emphasis was placed on the viability of urine as a matrix to facilitate at home collections from post-kidney transplant patients and correlate expected outcomes for the advanced exosome assay. This included the stability of exosomal mRNA in urine upon transportation from the point of collection to a centralized testing facility, the storage of urine samples at different conditions upon receipt until the point molecular

assay is performed, and the effect of various potentially interfering substances on the downstream quantitative polymerase chain reaction (qPCR) assay. mRNA from the urine-derived exosomes was shown to be stable in a broad range of conditions and produced accurate results when analyzed *via* qPCR assay for detection of kidney allograft rejection. We identified the most optimal conditions for every step of the process, ensuring preanalytical sample integrity and robust qPCR results.

MATERIALS AND METHODS

Specimen transportation boxes

The following box was utilized in studies on transportation of urine specimens: Small box cat #56519 (Therapak); exterior dimensions: 11 × 9 × 11 in; inner dimensions: 8 × 6 × 8 in; wall thickness: 1.5 in. Gel packs (12 oz Gel Pack #PP12 (Sonoco)) were frozen overnight at -20 °C and placed in the boxes, to refrigerate urine specimens. Temperature monitor #40510 (DeltaTrak) was used to monitor temperature inside the box, up to 72 h.

Isolation of Exosomes from the human urine samples

Exosome isolation was performed according to the ExoLution protocol (Exosome Diagnostics, a Bio-Techne brand). In brief, 10 mL urine samples were combined with 2.5 mL of 5×BB buffer in 15 mL tubes. ISS-MHV positive extraction control was added to each sample and loaded onto ExoLution Columns in 50 mL tubes and centrifuged for 5 min at 5000 × *g*. Flow-through was discarded and columns were washed with 10 mL of wash buffer and re-centrifuged at 5000 × *g*. ExoLution Columns were then transferred to fresh 50 mL tubes and 550 µL of lysis mix was added directly onto the ExoLution Column membrane surface. Columns were then centrifuged for 5 min at 5000 × *g*. Flowthrough containing exosomal RNA was collected and transferred to the RNA purification step.

RNA purification

RNA was further purified according to the ExoLution protocol using silica spin columns. Briefly, after a chloroform separation, the aqueous phase was transferred to 1.5 mL reaction tubes containing ethanol and loaded onto silica spin columns. Spin columns were centrifuged for 30 sec at 11000 × *g*. The flow-through was discarded and the spin columns were re-assembled onto the next set of fresh 2 mL collection tubes. The previous steps were repeated until the entire sample was loaded onto each column.

Next, RNA Prep buffer was added to the silica spin columns, and columns were centrifuged for 30 sec at 11000 × *g*. The flow-through was discarded, and the spin columns were re-assembled on the next set of fresh 2 mL collection tubes. RNA Wash buffer was next added to the spin columns, and columns were centrifuged for 30 sec at 11000 × *g*. The flow-through was discarded, and the spin columns were re-assembled on the next set of fresh 2 mL collection tubes. The wash process was then repeated a second time.

The spin columns were centrifuged for 3 min at 16000 × *g* to dry the membranes. The 2 mL collection tubes were discarded, and the spin columns were placed in fresh 1.5 mL reaction tubes. 15 µL of TE buffer was added directly onto the silica membranes. The columns were incubated for 5 min. Purified exosomal RNA was finally eluted by column centrifugation for 1 min at 16000 × *g* and immediately carried forward to reverse transcription.

Reverse transcription

Reverse transcription (RT) reaction mix was prepared using SuperScript® VILO™ cDNA Synthesis Kit (Thermo Fisher Scientific, cat# 11754-250): 10x SuperScript Enzyme Mix 2 µL, 5x VILO Reaction Mix 4 uL, exosomal RNA 14 µL. Samples were placed into thermocycler and were subject to the following cycling conditions: 25 °C: 10 min, 42 °C: 70 min, 85 °C: 5 min, hold: 4 °C.

Pre-amplification

Pre-Amplification was carried out as follows: TaqMan™ PreAmp Master mix (Thermo Fisher Scientific, 4488593): 12.5 uL, Primer mix 0.5 uL, RT reaction samples 12 uL. Samples were placed into thermal cycler and the following cycling parameters were utilized: Initial denaturation 95 °C for 10 min; 14 cycles: (95 °C for 15 sec, 60 °C for 4 min); hold: 4 °C.

qPCR

qPCR analysis was performed in triplicate, under the following conditions: TaqMan™ Fast Universal PCR Master Mix (Thermo Fisher Scientific, cat# 4367846): 10 µL, 20x assay mix: 1 µL[11], nuclease-free water: 7 µL, 5x diluted PreAmp sample: 2 µL. qPCR plates were analyzed on QuantStudio 5 (QS5) fast real-time PCR machine with 96-well 0.1 mL Block. Fast cycling conditions were utilized: initial denaturation 95 °C for 20 sec, 40 cycles: (95 °C for 1 sec, 60 °C for 20 sec).

Interference substances: Medications commonly prescribed to transplant patients

All pure drug compounds were acquired from Fisher Scientific: Teriflunomide (cat# AC467112500), Cyclosporin A (cat# AAJ6319106), Sirolimus (cat# AAJ62473MF), Everolimus (cat# AAJ60139MB), Mycophenolic acid (cat# AAJ6190509), Tacrolimus (cat# AAJ63571MF), Ascomycin (cat# AAJ66751MC), dissolved in DSMO to produce 5000× stocks, and tested for potential interference with assay at concentrations 50× exceeding expected urinary excretion level transplant patients. Expected levels: Tacrolimus: 0.53 µg/mL; Cyclosporin A: 10.5 µg/mL; Mycophenolic acid: 16.67 µg/mL; Everolimus: 0.03 µg/mL; Sirolimus: 0.10 µg/mL; Ascomycin: 0.47 µg/mL; Teriflunomide: 18.33 µg/mL.

RESULTS

Ideally, active surveillance testing for post-transplant monitoring should be facilitated through an at home non-invasive collection of the patients' specimens. Alternatively, the procedure could be performed with ease in a healthcare provider's office. Regardless of the point of collection, the specimen must be transported to a centralized laboratory, accessioned and temporarily stored prior to analysis.

The objective of this study was to explore stability of urinary exosomes and their diagnostic value with a previously described analytical method[14] for the detection and stratification of kidney allograft rejection. Briefly, in this assay the urine sample is first passed through the column to capture all extracellular vesicles, next the vesicles are lysed to release their content and the exosomal RNA is purified on the mini-column, reverse transcription is performed, next pre-amplification, followed by a qPCR analysis for a panel of mRNA markers. The output Ct values are analyzed by an algorithm that generates the scores that allow determination of rejection for kidney allograft, and type of rejection: TCMR *vs* ABMR. The entire assay takes less than 6 h to complete, which enables the laboratory to produce rapid "same day" results.

Taking into account the novelty of urine as a sample of choice for this molecular assay, and also exosomes, particularly their RNA cargo, as markers of organ rejection, it is of high importance to characterize every step in more detail, to ensure subsequent smooth clinical implementation of this test. Here we explored: (1) Stability of urine specimens and exosomal mRNA upon transportation from the point of collection to testing facilities, (2) storage of urine samples at different conditions upon receipt till the point molecular assay is performed, and (3) effect of various potentially interfering substances on the downstream qPCR assay.

Stability of exosomal mRNA upon urine transportation at different temperatures, and effects of various urine upfront processing techniques

Stability of exosomal mRNA upon preanalytical conditions mimicking urine transportation at different temperatures, and effects of various urine upfront processing techniques were studied in detail. Results are shown in Figure 1. Urine specimens were derived from 4 donors, and processed following the complete workflow, with qPCR assay performed for a panel of 9 mRNA targets. Preanalytical conditions mimicking specimen shipment and urine processing included: 2 d storage at +4 °C; 2 d at +20 °C; 2 d at +40 °C; whole urine frozen and thawed (mimicking transportation in frozen state); urine pre-processed to remove cells and debris by centrifugation for 20 min at 2000 × *g*, and supernatant subject to single or double freeze/thaw cycles.

Storage of urine samples at elevated temperature, +20 °C and especially +40 °C caused gradual degradation of mRNA, as indicated by Ct (threshold cycles) shift that varied among targets and donors (as expected). While for some donors and certain mRNA targets Ct shift was minimal (< 0.5 Ct), for other donors and certain mRNA targets effects were substantial (> 5 Ct). Importantly, variable increases in Ct values introduce unpredictable changes to the outputs of algorithms analyzing levels of multiple mRNA targets and as a consequence will decrease accuracy of calculated outcomes. Normalization to PGK1 or other RNA largely addresses this issue. However, overall it is recommended that urine specimens be transported refrigerated, to ensure temperatures are consistently maintained below +20 °C.

Freezing urine specimens on dry ice and -80 °C, followed by defrosting, caused mild increase in Ct values compared to unfrozen specimens, presumably due to partial mRNA degradation upon sample thawing; 2 freeze/thaw cycles did not have significant further effects on mRNA integrity.

Specimens which were frozen without initial centrifugation at low G-force to deplete cells and debris generated somewhat variable Ct values for some samples and mRNA targets. This indicates the need for preanalytical centrifugation of urine specimens to ensure removal of interfering cellular RNA fraction (including blood-derived: See discussion below), consistent qPCR results, and assay algorithmic output scores.

In summary, for exosome-based molecular assays, the preanalytical process plays an important role in downstream assay integrity. Urine specimens must be transported under refrigeration, and centrifugation of the samples ensures depletion of cells and debris, and consistent, reproducible results. Effects of urine freezing and prolonged storage on the accuracy and reproducibility of the assay will be further discussed below.

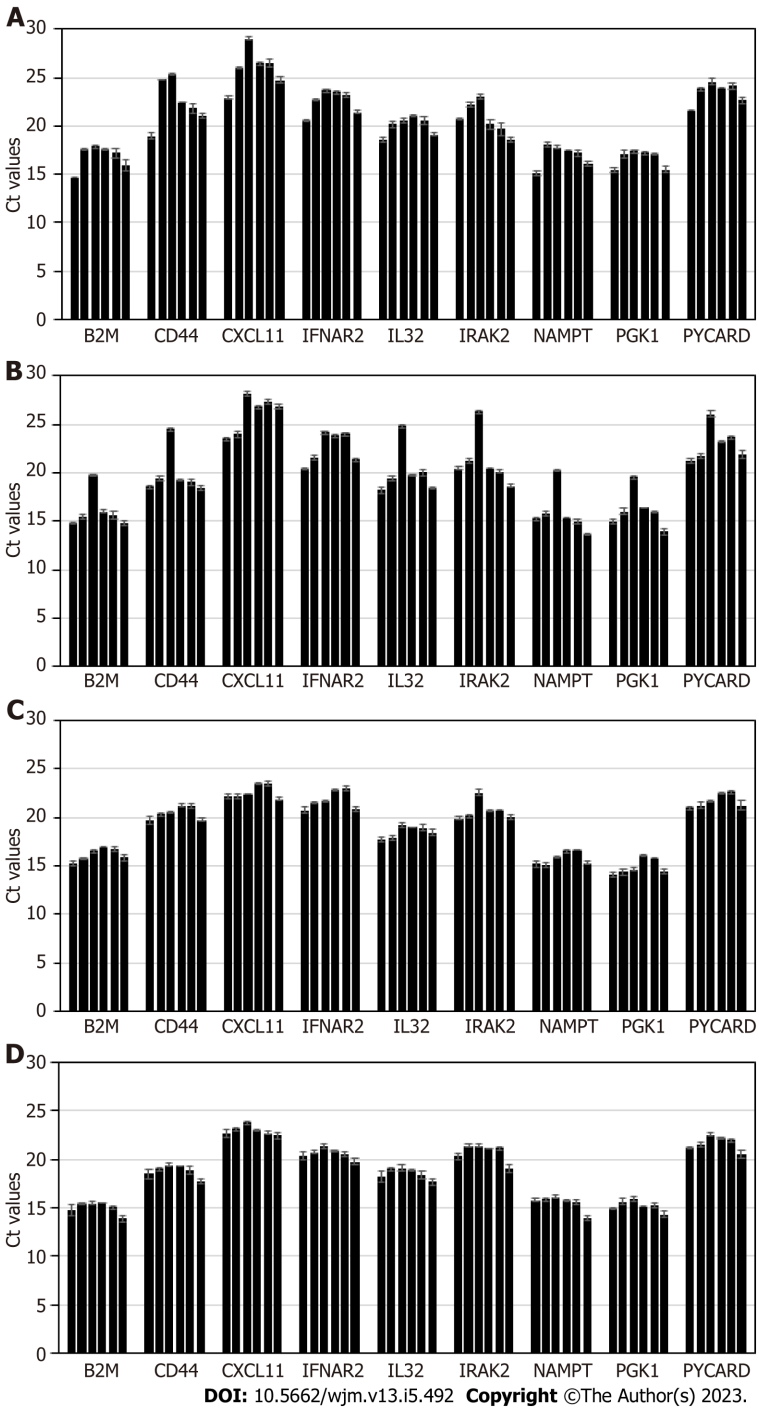
Next, temperature of the urine specimens was monitored during conditions mimicking transportation, to identify the optimal gel pack volume that would ensure sample refrigeration (Figure 2). One, two, three or four 12 oz gel packs were frozen overnight at -20 °C and placed in the 1.5-inch thick styrofoam box, that was stored at ambient temperature. Results are shown in Figure 2.

At 24 h, which is typical for overnight sample shipment, temperature inside the box with single gel pack (12 oz) was approximately 14 °C; two, three or four gel packs successfully maintained temperature below 4 °C. By 48 h, temperature inside the boxes with 1-3 gel packs (12 oz) was > 15 °C; only four gel packs secured temperature of approximately 8 °C. By 72h, temperature inside all boxes with 1-4 gel packs (12 oz) was > 18 °C.

Overall, for molecular tests, harvested urine specimens should be shipped by next day overnight delivery, in Styrofoam boxes ≥ 1.5 inch thick with gel packs totaling 24-48 oz, to ensure optimal refrigeration and specimen temperature for 24-48 h.

Stability of exosomal mRNA upon prolonged urine storage at different temperatures

Stability of exosomal mRNA was next studied upon prolonged urine storage at different temperatures. Results are shown in Figure 3. Urine samples were derived from 4 donors, and processed following the complete workflow, with qPCR assay performed for a panel of 9 mRNA targets. Conditions included: 2 d at +4 °C; 7 d at +4 °C; 14 d at +4 °C; 4 d at -80 °C; 30 d at -80 °C.

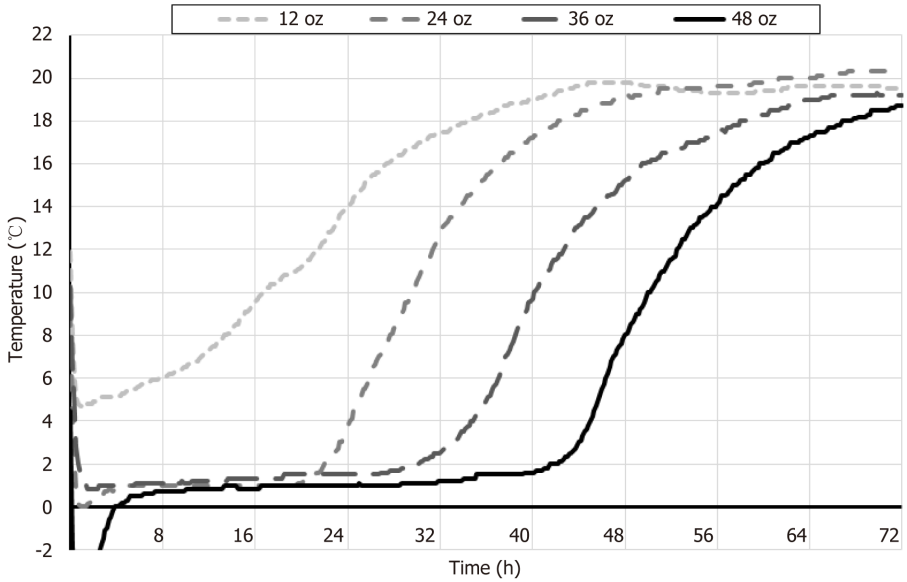


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Figure 1 Stability of exosomal mRNA upon urine transportation at different temperatures, and effects of various urine upfront processing techniques. A: Donor 1; B: Donor 2; C: Donor 3; D: Donor 4. For each donor, levels of 9 mRNA targets were analyzed by quantitative polymerase chain reaction; the graph displays Ct values (y-axis). Conditions mimicking urine transportation and handling, left to right: 2 d at +4C; 2 d at +20C; 2 d at +40C; freeze/thaw; 2x freeze/thaw; urine freeze/thaw without pre-processing.

Storage of urine samples at +4 °C for up to one week did not affect mRNA integrity and assay outcome; at 2 wk of storage gradual degradation of mRNA is occurring, as indicated by Ct shift that varied for different targets and donor-to-donor (as expected). Thus, unfrozen urine specimens should ideally be tested within 7 d of specimen receipt and refrigerated storage.

Once samples were frozen at -80 °C, and then defrosted and tested in assay, Ct values typically shift up compared to fresh samples, and for some donors and certain mRNA targets the change is significant (> 2 Ct). Prolonged sample storage at -80 °C does not cause any further Ct increase. Thus, for the purpose of consistency and reproducibility, all urine samples should be either processed unfrozen (within a week, as stated above) or all subject to centrifugation at low G-force, frozen, then defrosted and tested at any time point. As mentioned above, normalization to PGK1 or other RNA, will ensure consistent assay performance.



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Figure 2 Optimal refrigeration of urine specimens upon conditions mimicking shipment. One, two, three or four 12 oz gel packs were frozen overnight at -20C and placed in the 1.5 inch thick styrofoam box, to provide refrigeration for urine specimens. The graphs display temperature inside the box, over the range of 72 h.

Effect of interference substances on qPCR assay

First, medications commonly prescribed to transplant patients, were studied for potential interference with exosome molecular assay. Urine samples derived from 4 donors were utilized, and various parent drugs and metabolites were spiked in before exosome purification, at concentrations exceeding 50-fold the expected urinary excretion levels for transplant patients. Expected urine levels for immunosuppressants and other common medications were: Tacrolimus: 0.53 μg/mL; Cyclosporin A: 10.5 μg/mL; Mycophenolic acid: 16.67 μg/mL; Everolimus: 0.03 μg/mL; Sirolimus: 0.10 μg/mL; Ascomycin: 0.47 μg/mL; Teriflunomide: 18.33 μg/mL.

Samples were processed following the complete workflow, and qPCR assay was performed for a panel of 9 mRNA targets. Results are displayed in Figure 4. None of the drugs caused major interference with assay at the dose significantly exceeding the typical circulating levels in bodily fluids, as indicated by Ct values within +/- 1 compared to controls. This demonstrates that molecular assay is robust, and all common medications are depleted from the mRNA analytes during the exosome and RNA purification workflow, and the trace amounts remaining do not have negative impact on reverse transcription or real-time PCR.

Next, effect of trace amount of blood in urine was studied. Hematuria is a fairly common condition, and blood was expected to impact the performance of exosome molecular assay. Urine samples derived from 4 donors were utilized, and blood (0%-1%) was spiked into urine before exosome purification. Results are shown in Figure 5.

Blood in urine did not inhibit mRNA target detection but rather added its own "signal" (originating from blood-derived cellular mRNA) to that of the urinary exosomes. For all mRNA analytes, Ct values significantly decrease indicating several fold increase in mRNA levels due to blood-derived mRNA co-purifying with exosomal RNA. However, once the urine specimens are centrifuged (which is an obligatory part of the exosome assay, as was described above), the bulk of blood cells are successfully depleted and Ct values generated are very similar to normal urine specimens. Spiking in serum instead of blood confirmed the results - assay interference is coming from blood-derived components that can be successfully removed by centrifugation.

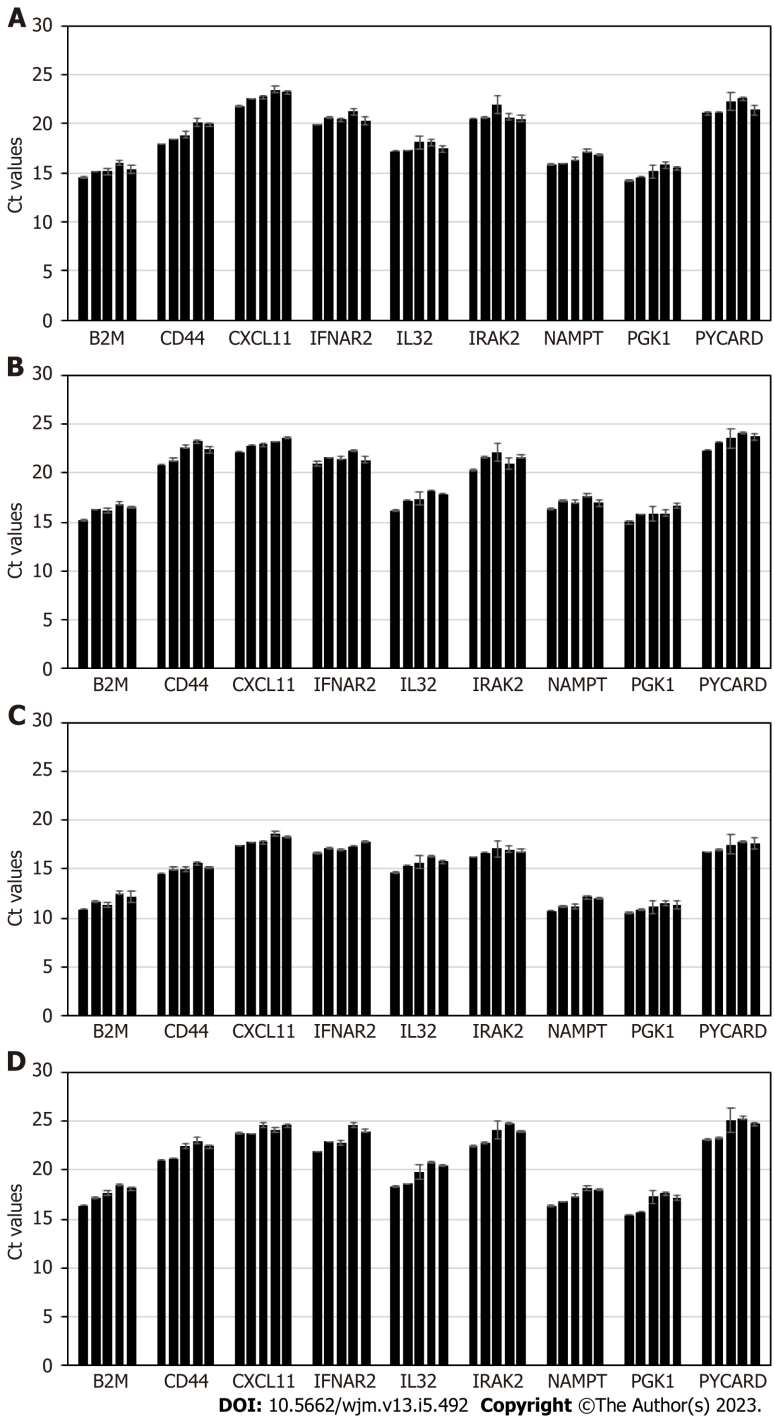
In case blood cells are lysed, the contents obviously cannot be easily spotted or removed from urine; however such RNA is rapidly degrading in urine that contains high levels of RNases, and thus interference with assays should be minimal – if any.

Overall, centrifugation of the urine samples ensures depletion of blood-derived cells and large debris, and consistent, reproducible results. As a precaution, urine samples with visible hematuria should be excluded for this molecular test.

DISCUSSION

Solid organ transplantation has made tremendous progress in the last decade- aided by imaging techniques, donor-recipient human leukocyte antigen matching, and immunosuppressive therapy. In the United States alone, more than 40000 organ transplants are performed annually, with kidney, liver, heart, and lung being among the most common.

Today, major challenges for transplantation are improving long-term graft viability and preserving patient quality of life. Allograft rejection represents the greatest risk of transplant failure among patients. Early identification of subclinical injury, and the subsequent differentiation of injury type, could enable earlier clinician intervention and provide

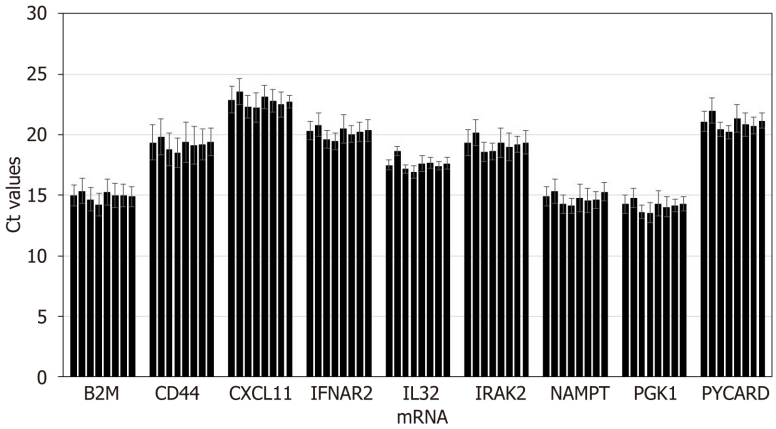


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Figure 3 Stability of exosomal mRNA upon prolonged urine storage at different temperatures. A: Donor 1; B: Donor 2; C: Donor 3; D: Donor 4. For each donor, levels of 9 mRNA targets were analyzed by quantitative polymerase chain reaction; the graph displays Ct values (y-axis). Urine storage conditions, left to right: 2 d at +4C; 7 d at +4C; 14 d at +4C; 4 d frozen at -80C; 30 d frozen at -80C.

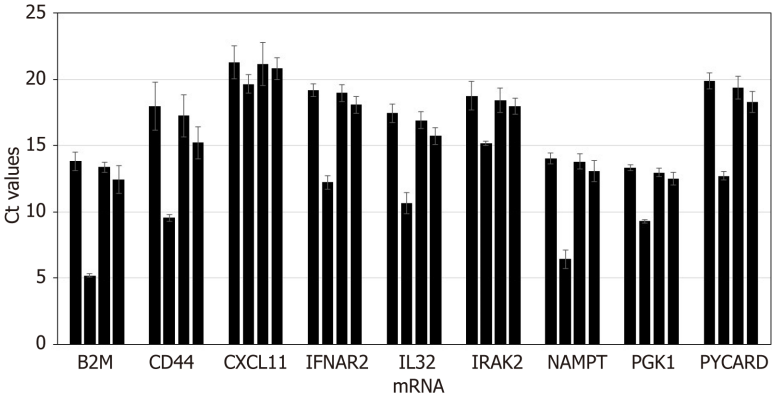
opportunities for personalized treatment. Further, minimally invasive methods of post-transplant monitoring could improve patients' quality of life while serving as a rejection screening tool and adjunct to histopathology.

One of the more promising discoveries in recent years has been the use of exosomal mRNA signatures to determine allograft health[14]. Urine has long been known as a valuable source of molecules serving as diagnostic markers for renal disease, and urinary exosomes have been shown to be effective screening tools for kidney allograft rejection. With herein demonstrated mRNA stability and overall robustness, urinary exosomal assays could represent a non-invasive and less burdensome approach to monitor graft health and also potentially open the door for at-home sample collections.



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Figure 4 Effect of common drugs on performance of the exosome molecular assay. Urine samples derived from 4 donors were utilized, and various drugs were spiked into urine before exosome purification, at levels exceeding the typical urinary concentrations by 50-fold. Nine mRNA targets were analyzed by quantitative polymerase chain reaction; the graph displays Ct values (y-axis). Drugs, left to right: Control, Tacrolimus, Mycophenolate, Cyclosporin A, Sirolimus, Everolimus, Teriflunomide, Ascomycin.



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Figure 5 Effect of blood on performance of the exosome molecular assay. Urine samples derived from 4 donors were utilized, and blood or serum was spiked into urine before exosome purification. Levels of 9 mRNA targets were analyzed by quantitative polymerase chain reaction; the graph displays Ct values (y-axis). Conditions left to right: control urine specimens, processed following the standard protocol; urine specimens spiked with 1% blood and processed without additional centrifugation; urine specimens spiked with 1% blood and pre-processed following the standard protocol (centrifugation for 20 min at 2000 × *g*); urine specimens spiked with 1% serum and processed without additional centrifugation.

CONCLUSION

To conclude, we characterized molecular assay utilizing urine-derived exosomes. This included stability of urine samples upon transportation from the point of collection to a centralized testing facility, storage of urine at different conditions upon receipt till the point molecular assay is performed, upfront processing, and effect of various interference substances on the downstream qPCR assay. mRNA from urine-derived exosomes was shown to be stable across a broad range of conditions and produced accurate results when analyzed *via* qPCR assay for kidney allograft rejection. We identified the most optimal conditions for every step of the process, ensuring preanalytical sample integrity and robust qPCR results.

ARTICLE HIGHLIGHTS

Research background

Exosomes are nano-sized extracellular vesicles with nucleic acid and protein cargo, actively secreted by all cells within human body, and found in abundance in all body fluids, including urine. These extracellular vesicles have tremendous potential for next generation diagnostics, theoretically enabling noninvasive assessment of organ and tissue function *via* liquid biopsy analysis.

Research motivation

Recently, feasibility of an exosomal molecular test was demonstrated for post-organ transplant monitoring: analysis of urine-derived exosomal mRNA cargo allowed early detection of kidney allograft rejection. Taking into account the novelty of this approach, urine and in particular extracellular vesicles with their diverse RNA cargo have to be better characterized to ensure robustness of this molecular assay.

Research objectives

We further studied urine-derived exosomes and their mRNA content as a highly promising diagnostic modality. This included stability studies of urine samples and exosomal mRNA upon transportation from the point of collection to a centralized testing facility, short-term storage of urine at different conditions upon receipt till the point molecular assay is performed, and effects of various potentially interfering substances on the downstream quantitative polymerase chain reaction (qPCR) assay.

Research methods

The urine specimens were stored at various temperatures and conditions and pre-processed in different ways. Next, samples were passed through the columns to capture all extracellular vesicles, the vesicles were lysed to release their content and the exosomal RNA was purified on the mini-columns, reverse transcription was performed, next pre-amplification, followed by a qPCR analysis for a panel of mRNA markers.

Research results

To ensure exosomal RNA integrity, the harvested urine specimens should be shipped refrigerated, by overnight delivery. Urine can next be stored at the test site for up to 1 wk at 4 °C, and long term should be frozen at -80 °C. Urine specimens must be centrifuged at low G-force to deplete cells and debris, to ensure consistent top results in downstream molecular assays. All commonly used medications (tacrolimus, cyclosporin A, mycophenolic acid, everolimus, sirolimus, ascomycin, teriflunomide) were tested and confirmed that they do not cause assay interference.

Research conclusions

mRNA from the urine-derived exosomes was proven to be stable across a broad range of conditions and produce robust results in molecular post-transplant monitoring assays. We identified optimal conditions for every step of the workflow, ensuring pre-analytical sample integrity and robust downstream qPCR results.

Research perspectives

Exosomes and in particular their mRNA cargo have the potential to revolutionize post-transplant monitoring, and detect early rejection events for kidney as well as other allografts- based on molecular analysis of urine, saliva and other body fluids.

FOOTNOTES

Author contributions: McFaul M, Ventura C, Evans S, Dundar H performed the experiments; Rumpler MJ, McCloskey C, Lowe D, Vlassov AV planned the study and wrote the manuscript.

Institutional review board statement: The study was reviewed and approved by Thermo Fisher Scientific.

Conflict-of-interest statement: Authors are employees of Thermo Fisher Scientific.

Data sharing statement: No additional data are available.

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ORCID number: Alexandre V Vlassov 0009-0000-9070-9870.

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CASE REPORT

Successful hip revision surgery following refracture of a modern femoral stem using a cortical window osteotomy technique: A case report and review of literature

Carlos M Lucero, Juan B Luco, Agustin Garcia-Mansilla, Pablo A Slullitel, Gerardo Zanotti, Fernando Comba, Martin A Buttaro

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Carlos M Lucero, Gerardo Zanotti, Fernando Comba, Martin A Buttaro, Centro de Cadera "Sir John Charnley", Instituto de Ortopedia y Traumatologia Prof. Dr. Carlos E. Ottolenghi, Hospital Italiano de Buenos Aires, Capital federal C1198AAW, Buenos Aires, Argentina

Juan B Luco, Instituto de Ortopedia y Traumatologia Prof. Dr. Carlos E. Ottolenghi, Hospital Italiano de Buenos Aires, Ciudad Autonoma de Buenos Aires C1198AAW, Buenos Aires, Argentina

Agustin Garcia-Mansilla, Pablo A Slullitel, Centro de Cadera "Sir John Charnley", Instituto de Ortopedia y Traumatología, "Prof. Dr. Carlos E. Ottolenghi", Hospital Italiano de Buenos Aires, Ciudad Autónoma de Buenos Aires C1198AAW, Buenos Aires, Argentina

Corresponding author: Juan B Luco, MD, Instituto de Ortopedia y Traumatologia Prof. Dr. Carlos E. Ottolenghi, Hospital Italiano de Buenos Aires, Peron 4190, Ciudad Autonoma de Buenos Aires C1198AAW, Buenos Aires, Argentina. bautista.luco@hospitalitaliano.org.ar

Abstract

BACKGROUND

The ExeterTM Universal cemented femoral component is widely used for total hip replacement surgery. Although there have been few reports of femoral component fracture, removal of a broken femoral stem can be a challenging procedure.

CASE SUMMARY

A 54-year-old man with a Dorr A femur sustained a refracture of a primary ExeterTM stem, two years after receiving a revision using a cement-within-cement technique (CWC) through an extended trochanteric osteotomy (ETO). The technical problems related to the CWC technique and the ETO played a major role in the stem fatigue refracture. We performed revision surgery and removed the distal cement using a cortical femoral window technique, followed by re-implantation with an uncemented, modular, distally-fixed uncemented stem. The patient experienced an uneventful postoperative recovery.

CONCLUSION

Re-fracture of a modern femoral ExeterTM stem is a rare event, but technical complications related to revision surgery can lead to this outcome. The cortical

window osteotomy technique can facilitate the removal of a broken stem and cement, allowing for prosthetic re-implantation under direct vision and avoiding ETO-related complications.

Key Words: Exeter stem; Femoral stem breakage; Femoral osteotomy; Total hip arthroplasty; Case seport

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Core Tip: We analyzed the causes of failure in a patient with an Exeter stem refracture, and discussed how to resolve it using a known but little used technique. When removing a broken stem, a window osteotomy facilitates the extraction of the distal cement and allows for prosthetic reimplantation, thereby minimizing the complications of an extended osteotomy. Finally, this preoperative technique, if correctly planned, can be performed by using ordinary instruments and does not consume host bone. This technique should be an addition to the armamentarium of a revision hip surgeon when faced with the challenge of extracting a fracture cemented femoral stem.

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INTRODUCTION

Femoral stem fracture is a rare complication in total hip replacement (THR). Over the years, the stems have been redesigned and failure has been greatly minimized because of improved designs, the use of modern materials, and better cementation techniques[1]. The ExeterTM stem (Stryker, Newbury, United Kingdom) has shown excellent long-term survival[2,3]. Fractures are an extremely rare complication with a rate of around 0.2%[4,5]. Fracture of this particular design has already been reported, and was mainly associated with the original stem in the 70s[6-9]. However, as far as we are concerned, presented here is the first case of a re-fracture of an ExeterTM stem in the same patient. Removal of a broken femoral component is a technically demanding procedure and several techniques have been described to treat this complication[10-14] and to preserve as much bone as possible. In this case report, we described a patient with an ExeterTM stem re-fracture, analyzed the possible causes of this complication and surgically managed the case by using a simple technique that aimed to preserve bone and the abductor mechanism.

CASE PRESENTATION

Chief complaints

The patient's chief complaint involves post-operative hip pain, with an absence of concomitant traumatic antecedents.

History of present illness

A 54-year-old man [170 cm, 90 kg, body mass index (BMI) 31.1 kg/m^2] was admitted to another center 7 years ago for a one-stage bilateral THR due to osteoarthritis.

Five years later, he started to develop pain in his right thigh without previous trauma and was diagnosed with a fracture in the mid-proximal third of the femoral stem (Figure 1A and B). On reviewing his postoperative note, an ExeterTM V40TM/TridentTM X3TM (Stryker, Newbury, United Kingdom) hybrid right THR was implanted through a postero-lateral approach, using a 44 mm stem and a 50 mm cup was placed with a 32 mm ceramic (alumina) BioloxTM Forte (CeramTec, Plochingen, Germany) femoral head and a 10 highly cross-linked polyethylene (HXLPE). At revision surgery, the broken femoral stem was removed using an ETO, and a new ExeterTM V40TM stem was implanted by employing a CWC using the same stem size as the first intervention (Figure 1C). The patient evolved favorably without pain and returned to his functional status. Two years after revision the patient came to our institution, presenting pain in the operated limb, without reporting any associated trauma. Antero-posterior (AP) X-ray of both hips was taken, showing a new fracture of the right stem at the level of the mid-distal third, without apparent bone fracture and with the distal cement mantle undamaged (Figure 1D). The patient was scheduled for a femoral revision with a cement-less distal fixation stem. A minimally invasive window on the lateral cortex of the femur was made, to extract the cement mantle and the distal part of the fractured stem.

History of past illness

The information in question was not required for the case report.

Personal and family history
The information in question was not required for the case report.

Physical examination
Two years after revision the patient came to our institution, presenting pain in the operated limb, without reporting any associated trauma.

Laboratory examinations
The information in question was not required for the case report.

Imaging examinations
AP X-ray of both hips was taken, showing a new fracture of the right stem at the level of the mid-distal third, without apparent bone fracture and with the distal cement mantle undamaged (Figure 1D).

FINAL DIAGNOSIS
New fracture of the right stem at the level of the mid-distal third, without apparent bone fracture and with the distal cement mantle undamaged.

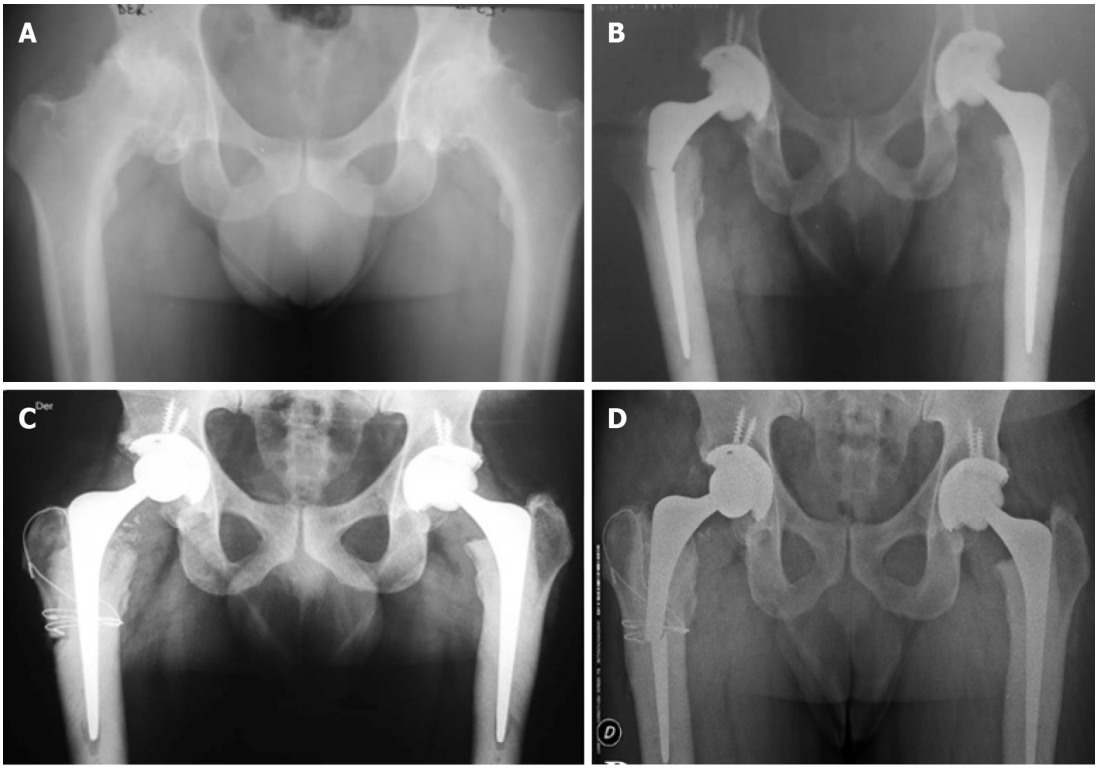
TREATMENT
Surgical technique
The following technique provides a method of "windowing" the femur, facilitating cement removal and firm fixation of the new prosthesis, in this case, an uncemented distally-fixed, RestorationTM Modular stem (Stryker, Newbury, United Kingdom). Preoperative radiographs must include an AP pelvic radiography covering both hips. Femoral radiographs should include the whole bone in AP as well as lateral (L) views. Calibration of the image is recommended in order to detect Dorr A narrow femoral canals like this one in which an uncemented, distally-fixed stem may not easily fit. In this case we used the known diameter of the failed femoral head, which was 32 mm, to calibrate the radiographs.

The patient was placed on the lateral decubitus position, with two anterior and one posterior supports in order to keep the pelvis stable and well-oriented during the whole surgery. A postero-lateral approach was used. The length was calculated with the previous preoperative planning on the radiography, measuring the distance from the tip of the greater trochanter to the end of the cement and the cement restrictor. The fascia lata and gluteal fascia were divided in line with skin incision. The fibers of the gluteus maximus were separated bluntly in line with skin incision. The sciatic nerve was then identified and protected.

When the patient reported a history of more than one revision, the fascia might have been scarred with the vastus externus, and dissection should be performed to separate these two different structures. Once the plane has been divided, a Charnley retractor was positioned, taking both sides of the fascia lata. The proximal cement and the proximal part of the fractured stem were removed with specially-designed chisels that had to be inserted between the cement and the bone, and then the cement was gently smashed with a light hammer (Figure 2A). All the proximal cement that was under direct vision was removed proximally, leaving the unseen polymethylmethacrylate and the cement restrictor to be resected through the cortical window. Direct vision of the removal prevented a cortical femoral perforation that could lead to an intraoperative femoral fracture. All necrotic tissues, pseudomembranes end cement were removed in order to maximize contact between the uncemented revision stem and the host bone. A cement chisel was introduced into the femoral canal, with the leg in the femoral position in order to measure the length of the cement where the tip of the stem was implanted. The vastus lateralis was detached and the cortical window was drawn with a surgical marker (Figure 2B). The cortical window could be made laterally or anterolaterally. The first option makes it easier for the surgeon to perform the windowing and to remove cement under direct vision. The second option has been advocated by some authors for its benefit of a lower incidence of postoperative femoral fracture[16]. But in all the cases this window is indicated, a longer stem bypassing at least 2 diaphyseal femoral diameters must be implanted.

The cortical window was made with a narrow saw in order to achieve maximal control of the cut (Figure 2B) and then it was detached with gentle chiseling (Figure 2C). The window was kept in a soaked swab until the femoral reconstruction was complete. Once the cement mantle was partially removed with a cement chisel, the fractured distal part of the cemented stem could be observed under direct vision. The next step was to impact the fractured stem from its tip in order to remove it retrogradely (Figure 2D). The window also allows for direct vision of the cement mantle as well as the cement restrictor. The distal part of the Exeter stem was removed. It is important to notice that this Exeter stem has been deficiently cemented proximally, and well-cemented distally, but the surgeon forgot to use the centralizer, which is of utmost importance for the proper functioning of this implant.

Femoral broaching was performed in order to achieve good contact between the host bone and the distal fixation stem. After this, the definitive conical distal stem was impacted into the femoral canal as preoperatively planned, under direct vision through the window, to achieve rotational stability and prevent subsidence (Figure 3A and B). Proximal cone reaming was then performed to prepare for the cone body placement. A BioloxTM Delta (CeramTec, Plochingen, Germany) 32 mm ceramic femoral head was implanted, retaining the highly cross-linked polyethylene, which was in



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Figure 1 X-ray. A: Antero-posterior preoperative X-ray of both hips where severe bilateral osteoarthritis is observed. A marked narrow endomedular canal is observed; B: Anteroposterior X-ray of both hips 5 years after operation, showing rupture at the proximal middle third level of the femoral stem; C: Immediate postoperative X-ray, showing femoral revision surgery. Trochanteric osteotomy and a poor proximal cement mantle are shown; D: Anteroposterior X-ray of both hips, 2 years after revision surgery, showing a further rupture of the Exeter® stem, at the level of prior femoral osteotomy.

good condition. Finally, reduction, stability test and limb length measurement were performed. Once procedure was finished, the cortical window was covered and fixed with wire cerclages (Figure 3C). Final postoperative AP X-rays exhibited excellent implant alignment, and the femoral windows was correctly bypassed with the new implant (Figure 3D).

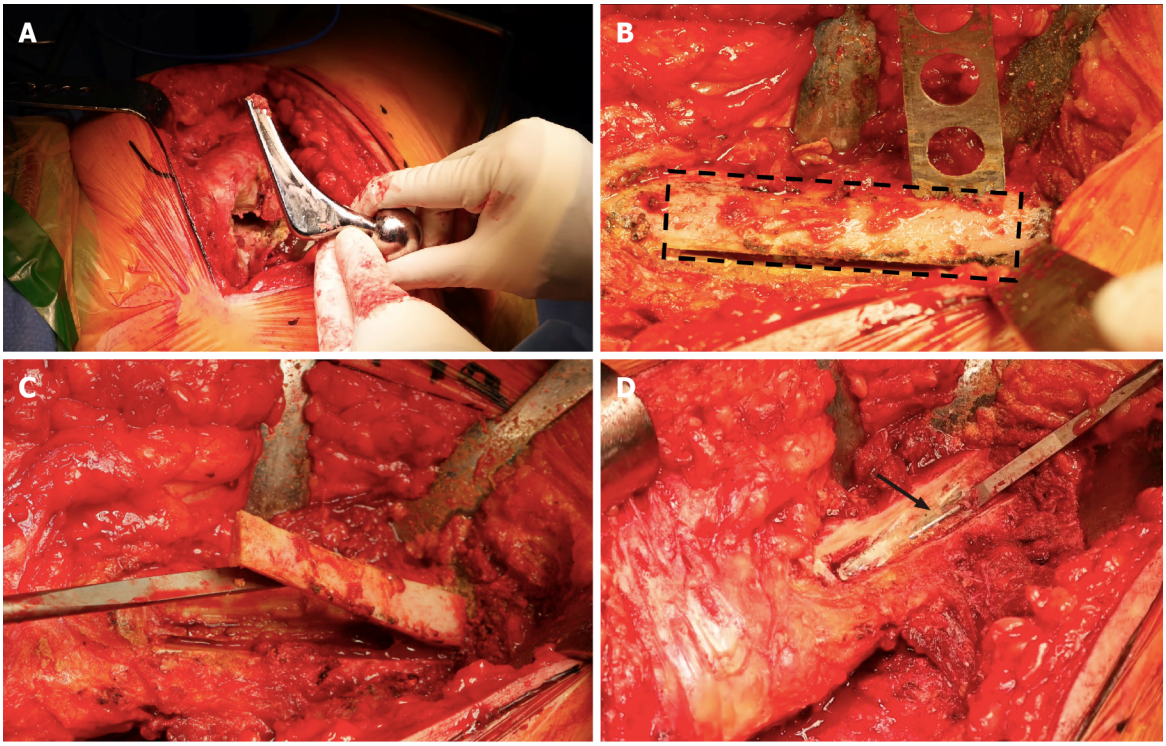
OUTCOME AND FOLLOW-UP

Postoperative, routine exercises included isometric exercises of the lower limbs and ambulation with a walker was allowed as tolerated from the first postoperative day. 48 h later, the patient was discharged home, with good pain tolerance and without clinical complications. Six months after operation, the patient had no limitations in activities of daily living. The most recent radiographic control showed stable prosthetic implants in correct position, and complete healing of the window osteotomy (Figure 4A and B).

DISCUSSION

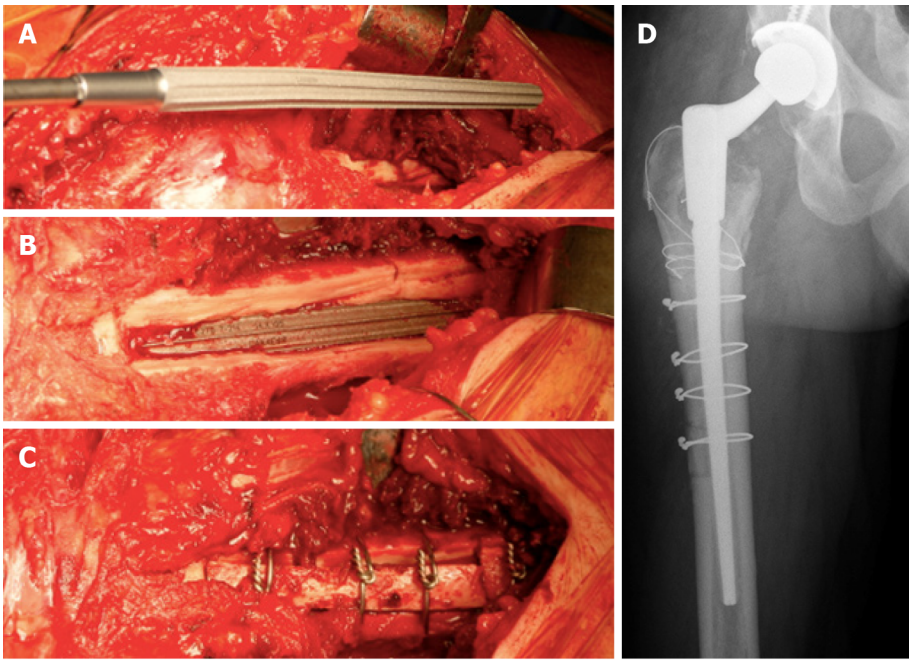
This case report describes two consecutive mechanical complications of a polished tapered high offset stem in a 54-year-old man having receiving operation in another center. The patient had a BMI > 30 and a femur with a Dorr A narrow medullary canal. Of note, a revision surgery was performed by employing a similar surgical technique, adding an ETO and using the CWC technique, and inserting an implant with the identical characteristics, that led to a refracture of the femoral stem. In our institution, the patient was revised to an uncemented distal fixation modular prosthesis, by removing the fractured stem through a femoral cortical window, allowing immediate full load as tolerated. The benefits of cortical windows are that this technique allows for easy removal of distal cement mantle or, as in this case, also the distal part of a broken cemented stem. Moreover, the osteotomized part can be easily fixed with some cerclage wires, it does not compromise the abductor mechanism, the patient has no restrictions to prevent a damage of this mechanism, and the stem can be fixed to an intact major trochanter, which is not possible when an ETO is performed. Furthermore, an ETO could have led to pseudoarthrosis, lack of proximal support and a new stem fracture.

In certain cases, as in Dorr A femurs, primary hip arthroplasty can be challenging. These femurs have strong thick cortices where uncemented fixation could be related to incomplete fitting of the stem because this one gets stuck in the proximal diaphysis before metaphyseal fixation occurs. Short partial neck preserving stems corresponding to the type 2B



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Figure 2 Surgical operation. A: Intraoperative images of implant removal during re-revision surgery, showing the removal of the proximal part of the broken femoral stem; B: Planning and execution of lateral or window femoral osteotomy (black dotted line) with oscillating saw; C: Osteotomized segment of the window and exposure of the medullary canal; D: Removing the cement and distal segment of the stem (black arrow).



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Figure 3 Femoral broaching was performed in order to achieve good contact between the host bone and the distal fixation stem. A: Intraoperative images during re-revision surgery. Definitive cementless distal fixation modular stem; B: Direct view of the insertion of the new stem, through the femoral window; C: Osteosynthesis of the osteotomized segment with wires; D: A femur Anteroposterior X-ray, where the final immediate postoperative result is shown with an uncemented distal fixation femoral stem and osteosynthesis of the cortical window.



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Figure 4 Antero-posterior and lateral X-ray. A: Lateral X-ray; B: Antero-posterior X-ray. Antero-posterior and lateral X-ray 3 mo after re-revision surgery, in which the correct position of the femoral implant and consolidation of osteotomy are shown.

of the Khanuja *et al*[17] classification, could be an alternative in young patients with Dorr A femurs, since the fixation is more proximal and the femoral canal could be less invaded[18].

Since the introduction of the ExeterTM Universal Stem in 1988, there have been many reports on stem fracture, with the cause being multifactorial[19-25]. Factors such as poor medial support, insufficient proximal cement mantle, varus orientation of the stem, and increased patient weight (or a combination of these factors) have been considered. Fracture of the femoral component of the cemented total hip prosthesis is a rare but documented complication[26], with incidence ranging from 0.23% to 10.7%. In the current case, implantation of an under-sized stem, usually in a "champagne glass" femur, contributed to fracture in the body of the prosthesis. Although the fracture of a polished conical femoral stem is a rare event, a defect in the cementation technique can lead to a higher stress and suffices to increase the risk of fatigue rupture even in non-obese patients. Moreover, as reported by O'Neill *et al*[27], the cement-in-cement revision could also predispose stems to the breakage of a polished stem, probably because the the new stem must be proximally implanted, with less cement mantle, which would lead to poor metaphyseal fixation, thus generating a probable cantilever effect. For this reason, the 125 mm Exeter 44 mm offset stem was developed specifically for cement-in-cement revision, being 25 mm shorter than the standard stem and narrower in the distal and antero-posterior directions.

One of the main technical problems with a broken femoral stem is the removal of the distal part and its cement mantle. ETO is a popular and reproducible technique that can resolve most complications in a complicated THR, such as infection, aseptic loosening or a periprosthetic fracture. However, it is not without complications, and nonunion, subsidence, and trochanteric migration with subsequent Trendelenburg, at considerable rates, have been described[28-31]. The time to healing of the osteotomy and the restriction on immediate postoperative weight-bearing are considerable aspects when executing it. Several reports have described multiple methods to facilitate cement extraction and minimize complications during the procedure[32,33]. However, most of them require a protracted surgical time and may be associated with perforations of the femoral cortices or inadvertent intraoperative fractures[34].

Nelson *et al*[35] developed the original cortical window technique in 1980, creating a window on the lateral aspect of the femur. Klein *et al*[36], in a series of 21 THR revisions using a window made in the antero-lateral aspect of the femur to extract the distal cement, attained good results with an osteotomy consolidation rate of 17 wk, without thigh pain or loosening. The benefits of cortical windows are that this technique allows for easy removal of distal cement or, as in this case, also the distal part of a broken cemented stem. Moreover, the osteotomy can be easily fixed with two or three cerclage wires, it does not compromise the abductor mechanism, the patient has no restrictions to prevent the damage of this mechanism, and the stem can be fixed to an intact major trochanter, which is not possible when ETO is performed. Although preoperative planning of the window length is mandatory, it is possible to extend it if the surgeon needs to.

CONCLUSION

We analyzed the causes of failure in a patient with an Exeter stem refracture, and discussed how to resolve it using a known but little used technique. When removing a broken stem, a window osteotomy facilitates the extraction of the distal cement and allows for prosthetic reimplantation, thereby minimizing the complications of an extended osteotomy. Finally, this preoperative technique, if correctly planned, can be performed by using ordinary instruments and does not consume host bone. This technique should be an addition to the armamentarium of a revision hip surgeon when faced with the challenge of extracting a fracture cemented femoral stem.

FOOTNOTES

Author contributions: Garcia-Mansilla A collected the images and was a major contributor to the manuscript; Luco JB obtained consent from the patient and contributed to the editing of the manuscript; Buttaro M contributed to the editing of the manuscript and was the main supervisor of the project; All authors read and approved the final manuscript.

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Country/Territory of origin: Argentina

ORCID number: Carlos M Lucero 0000-0002-0833-7818; Juan B Luco 0009-0000-7551-2623; Agustin Garcia-Mansilla 0000-0001-9820-8886; Pablo A Slullitel 0000-0002-8957-075X; Gerardo Zanotti 0000-0001-8090-4832; Fernando Comba 0000-0002-2848-2983; Martin A Buttaro 0000-0003-3329-778X.

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