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Peer Reviewer of *World Journal of Experimental Medicine*, Xavier G Thomas, MD, PhD, Associate Professor, Hematology Department, Lyon-Sud University Hospital, 265 chemin du Grand-Revoyet, Pierre-Benite, 69495, France, xavier.thomas@chu-lyon.fr

AIMS AND SCOPE

The primary aim of the *World Journal of Experimental Medicine* (*WJEM*, *World J Exp Med*) is to provide scholars and readers from various fields of experimental medicine with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJEM mainly publishes articles reporting research results and findings obtained in the field of experimental medicine and covering a wide range of topics including clinical laboratory medicine (applied and basic research in hematology, body fluid examination, cytomorphology, genetic diagnosis of hematological disorders, thrombosis and hemostasis, and blood typing and transfusion), biochemical examination (applied and basic research in laboratory automation and information system, biochemical methodology, and biochemical diagnostics), *etc*.

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LETTER TO THE EDITOR

Fertility outcomes following bariatric surgery

Wassan Nori, Wisam Akram, Eham Amer Ali

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Wassan Nori, Department of Obstetrics and Gynecology, Mustansiriyah University, Baghdad 10052, Saydyia, Iraq

Wisam Akram, Department of Obstetrics and Gynecology, Mustansiriyah, Baghdad 10052, Al Yarmouk, Iraq

Eham Amer Ali, Department of Chemistry and Biochemistry, Mustansiriyah, Baghdad 10052, Iraq

Corresponding author: Wassan Nori, PhD, Academic Editor, Academic Research, Senior Researcher, Department of Obstetrics and Gynecology, Mustansiriyah University, Al -amin, Baghdad 10052, Saydyia, Iraq. dr.wassan76@uomustansiriyah.edu.iq

Abstract

Obesity impacts human health in more than one way. The influence of obesity on human reproduction and fertility has been extensively examined. Bariatric surgery (BS) has been used as an effective tool to achieve long-term weight loss in both sexes. BS improves hormonal profiling, increasing the odds of spontaneous pregnancy and success rates following assisted reproductive techniques in infertile females. For obese males, BS does improve sexual function and hormonal profile; however, conflicting reports discuss reduced sperm parameters following BS. Although the benefits of BS in the fertility field are acknowledged, many areas call for further research, like choosing the safest surgical techniques, determining the optimal timing to get pregnant, and resolving the uncertainty of sperm parameters.

Key Words: Bariatric surgery; Male fertility; Female fertility; Assisted reproduction technique; Seminal fluid

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Core Tip: One of the main strengths of bariatric surgery (BS) is achieving long-term weight reduction; many of the medical and dietary interventions have transient and sometimes ineffective results. Infertility is linked with obesity in many ways, including reducing the quality of produced gametes, disturbing the hormonal profile, and increasing oxidative stress, which in turn inversely affects many steps of human reproduction. BS can improve the fertility odds for both genders with assisted reproductive technique; additionally, it improves the odds of natural pregnancy.

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TO THE EDITOR

With interest, we read Kheirvari's study published in *World J Exp Med* 2022 about how obesity implicates organ transplants and the predictors of negative outcomes after bariatric surgery (BS)[1]. BS is an underused tool, serving about 1% to 2% of the populace. A misunderstanding about BS sometimes overshadows efforts to educate the general public on its advantages[1,2].

While the obesity epidemic endangers lives by escalating cardiovascular, metabolic, and malignant diseases, one of obesity's important associations is infertility[3].

Obesity is linked to gonadal dysfunction, resulting in female and male infertility at rates of 36% and 64%, respectively. Hyperandrogenism manifests in women as polycystic ovarian syndrome (PCOS). In contrast, hypoandrogenemia promotes male obesity-related secondary hypogonadism[4-6].

The capacity of BS to accomplish significant and long-lasting weight loss and the development of safer techniques are important factors that contribute to the fast growth of BS in fertility, which was not addressed in the study by Kheirvari *et al*[1].

A growing body of evidence confirms that reducing weight through BS may correct menstrual irregularity and ovulatory dysfunction, and increase spontaneous conception, especially among females with PCOS. Infertile obese women could conceive in 69% of the cases within two and a half years of follow-up[7-9]. The body mass index and the weight lost are independent predictors of pregnancy odds. Additionally, bariatric surgery increases live birth rates through assisted reproductive techniques[10].

Poor quality of life (QOL) hinders pregnancy odds and inversely impacts pregnancy outcomes. Furthermore, QOL is essential for evaluating nursing standards among infertile couples[11]. Obese couples have higher anxiety and depression caused by patients' external appearance added to the obesity-related pathology[11,12]. BS can improve patients' perspectives and help to restore the deranged hormonal profile[7-9]. Therefore, as part of their multidisciplinary approach, including the QOL score is recommended for follow-up of BS cases[13,14].

Although the current evidence supports the advantage of BS in female fertility, certain concerns have been raised about its safety. To begin with, the ideal surgical procedure for improving reproductive outcomes at a young age has yet to be established because BS involves numerous techniques. Furthermore, there is no strong consensus on the optimal time to conceive following BS. The problem of the decreased ovarian reservoir in older women continues to be debated[9,15].

Some but not all obese men have fertility issues; there are conflicting reports regarding the unfavorable impact on sperm parameters (SP), while others suffer erectile dysfunction. A state of hypogonadism was reported in obese men due to reduced sex hormone-binding globulin triggered by hyperinsulinemia[16]. BS in obese men may improve sexual activity and restore the hormonal profile, yet the effect on SP is conflicting, which calls for longitudinal study in that area[17]. Some reports declare reduced SP and assisted reproductive technique success rates following BS owing to nutritional deficiency after the surgery. However, others reported no change in SF up to one year following BS[18]. The concerns of worsening SF have resulted in raised sperm cryopreservation needs for selected cases [19].

In conclusion, BS appears to be an effective long-term weight-loss solution that can improve human well-being and QOL. The role of BS in infertility has been established; however, safety concerns, the surgical technique used, and the long-term effect on neonates and childhood are yet to be established. As for males, the inconsistency regarding regression in semen parameters necessitates a longitudinal study type to be resolved.

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Country/Territory of origin: Iraq

ORCID number: Wassan Nori 0000-0002-8749-2444; Wisam Akram 0000-0002-1968-7004; Eham Amer Ali 0000-0003-0559-9406.

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REFERENCES

1 **Kheirvari M**, Goudarzi H, Hemmatizadeh M, Anbara T. Bariatric surgery outcomes following organ transplantation: A review study. *World J Exp Med* 2022; **12**: 92-99 [PMID: 36196436 DOI: 10.5493/wjem.v12.i5.92]
2 **Gagner M**. Comment on: Conversion of laparoscopic sleeve gastrectomy to Roux-en-Y gastric bypass: patterns predicting persistent symptoms after revision. *Surg Obes Relat Dis* 2021; **17**: 1689-1690 [PMID: 34284960 DOI: 10.1016/j.soard.2021.06.018]
3 **Frank JW**. Controlling the obesity pandemic: Geoffrey Rose revisited. *Can J Public Health* 2022; **113**: 736-742 [PMID: 35451744 DOI: 10.17269/s41997-022-00636-6]
4 **Roomi AB**, Nori W, Al-Badry SH. The Value of Serum Adiponectin in Osteoporotic Women: Does Weight Have an Effect? *J Obes* 2021; **2021**: 5325813 [PMID: 34796028 DOI: 10.1155/2021/5325813]
5 **Charalampakis V**, Tahrani AA, Helmy A, Gupta JK, Singhal R. Polycystic ovary syndrome and endometrial hyperplasia: an overview of the role of bariatric surgery in female fertility. *Eur J Obstet Gynecol Reprod Biol* 2016; **207**: 220-226 [PMID: 27773356 DOI: 10.1016/j.ejogrb.2016.10.001]
6 **Wood GJA**, Tiseo BC, Paluello DV, de Martin H, Santo MA, Nahas W, Srougi M, Cocuzza M. Bariatric Surgery Impact on Reproductive Hormones, Semen Analysis, and Sperm DNA Fragmentation in Men with Severe Obesity: Prospective Study. *Obes Surg* 2020; **30**: 4840-4851 [PMID: 32700180 DOI: 10.1007/s11695-020-04851-3]
7 **Vitek WS**, Hoeger KM. Worth the wait? *Fertil Steril* 2022; **118**: 447-455 [PMID: 36116798 DOI: 10.1016/j.fertnstert.2022.07.001]
8 **Wilson R**, Aminian A, Tahrani AA. Metabolic surgery: A clinical update. *Diabetes Obes Metab* 2021; **23** Suppl 1: 63-83 [PMID: 33621412 DOI: 10.1111/dom.14235]
9 **Soares Júnior JM**, Lobel A, Ejzenberg D, Serafini PC, Baracat EC. Bariatric surgery in infertile women with morbid obesity: definitive solution? *Rev Assoc Med Bras (1992)* 2018; **64**: 565-567 [PMID: 30365653 DOI: 10.1590/1806-9282.64.07.565]
10 **Grzegorczyk-Martin V**, Fréour T, De Bantel Finet A, Bonnet E, Merzouk M, Roset J, Roger V, Cédrin-Durnerin I, Wainer R, Avril C, Landais P. IVF outcomes in patients with a history of bariatric surgery: a multicenter retrospective cohort study. *Hum Reprod* 2020; **35**: 2755-2762 [PMID: 33083823 DOI: 10.1093/humrep/deaa208]
11 **Cheng CY**, Stevenson EL, Yang CT, Liou SR. Stress and Quality of Life for Taiwanese Women Who Underwent Infertility Treatment. *J Obstet Gynecol Neonatal Nurs* 2018; **47**: 498-508 [PMID: 29715441 DOI: 10.1016/j.jogn.2018.03.005]
12 **Nori W**, Shallal F, Zghair MAG. Aspirin effect on Mid luteal Phase Doppler Indices in Patients with Recurrent Pregnancy Loss. *Int J Pharm Res* 2020; **12**: 2929-2934 [DOI: 10.31838/ijpr/2020.12.03.413]
13 **Ni Y**, Tong C, Huang L, Zhou W, Zhang A. The analysis of fertility quality of life and the influencing factors of patients with repeated implantation failure. *Health Qual Life Outcomes* 2021; **19**: 32 [PMID: 33494768 DOI: 10.1186/s12955-021-01666-3]
14 **Boivin J**, Takefman J, Braverman A. The Fertility Quality of Life (FertiQoL) tool: development and general psychometric properties. *Fertil Steril* 2011; **96**: 409-415.e3 [PMID: 21458806 DOI: 10.1016/j.fertnstert.2011.02.046]
15 **Parent B**, Martopullo I, Weiss NS, Khandelwal S, Fay EE, Rowhani-Rahbar A. Bariatric Surgery in Women of Childbearing Age, Timing Between an Operation and Birth, and Associated Perinatal Complications. *JAMA Surg* 2017; **152**: 128-135 [PMID: 27760265 DOI: 10.1001/jamasurg.2016.3621]
16 **Practice Committee of the American Society for Reproductive Medicine**. Obesity and reproduction: a committee opinion. *Fertil Steril* 2015; **104**: 1116-1126 [PMID: 26434804 DOI: 10.1016/j.fertnstert.2015.08.018]
17 **Reis LO**, Zani EL, Saad RD, Chaim EA, de Oliveira LC, Fregonesi A. Bariatric surgery does not interfere with sperm quality--a preliminary long-term study. *Reprod Sci* 2012; **19**: 1057-1062 [PMID: 22534335 DOI: 10.1177/1933719112440747]
18 **Sermondade N**, Massin N, Boitrelle F, Pfeffer J, Eustache F, Sifer C, Czernichow S, Lévy R. Sperm parameters and male fertility after bariatric surgery: three case series. *Reprod Biomed Online* 2012; **24**: 206-210 [PMID: 22196889 DOI: 10.1016/j.rbmo.2011.10.014]
19 **Reis LO**, Dias FG. Male fertility, obesity, and bariatric surgery. *Reprod Sci* 2012; **19**: 778-785 [PMID: 22534334 DOI: 10.1177/1933719112440053]



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LETTER TO THE EDITOR

4 Melatonin's actions are not limited to sleep

Venaki E, Koukkou E, Ilias I

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Peer Reviewer of *World Journal of Experimental Medicine*, Hala Gali-Muhtasib, PhD, Professor, American University of Beirut, Department of Biology, PO Box 11-0236, Riad El Solh 1107 2020, Beirut, Lebanon. amro@aub.edu.lb

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LETTER TO THE EDITOR

Melatonin's actions are not limited to sleep

Evangelia Venaki, Eftychia Koukkou, Ioannis Ilias

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Evangelia Venaki, Eftychia Koukkou, Ioannis Ilias, Department of Endocrinology, Diabetes and Metabolism, Elena Venizelou Hospital, Athens GR-11521, Greece

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

Abstract

Melatonin is widely available as a supplement, usually for sleep disorders. The consumption of melatonin supplements has increased considerably in recent years. An overlooked aspect of melatonin's administration is the resulting increase in prolactin secretion, *via* its action on hypothalamic dopaminergic neurons. We believe that since the effect of melatonin on prolactin is tangible, the laboratory finding of hyperprolactinemia could be encountered more often, given the increase in melatonin's use. This is an issue that merits further study.

Key Words: Melatonin; Sleep; Prolactin; Human; Dopamine; Side-effects

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Core Tip: Melatonin, although being an active hormone, is widely available as a supplement. The consumption of melatonin supplements has increased considerably in recent years. Melatonin may increase prolactin. The laboratory finding of hyperprolactinemia could be encountered more often, given the increase in melatonin's use.

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TO THE EDITOR

Melatonin, although being an active hormone, is widely available as a supplement[1-3]. It is taken to treat sleep disorders/insomnia, for adults usually at doses ranging from 0.5 to 6.0 mg at bedtime, with reported various degrees of effectiveness[4]. Other potential beneficial effects of melatonin include the prevention of delirium in hospit-

alized patients and - still at the experimental stage - restorative action on the cirrhotic liver[5,6]. Very recently, melatonin, given at 5.0 mg at bedtime in healthy older adults was considered to be effective in increasing sleep time and efficiency[7].

The consumption of melatonin supplements has increased considerably in recent years, as shown in studies in the United Kingdom and the United States[1,2]. In the United States, in the years 1999-2000, 0.4% of the population were taking melatonin, whereas in 2017-2018, this percentage rose to 2.1%[1]. An overlooked aspect of melatonin's administration is the resulting increase in prolactin secretion, *via* its action on hypothalamic dopaminergic neurons[8,9]. The relevant studies date to the previous century and in the most recent ones, melatonin given at a dose of up to 5 mg at bedtime quadrupled acutely prolactin levels compared to baseline[8,9].

Hyperprolactinemia is not uncommon, especially in women with polycystic ovaries syndrome, pituitary adenomas, hypothyroidism, macroprolactinemia, or therapy with hyperprolactinemia-inducing medications[10]. Interestingly, and despite the known action of melatonin on prolactin, melatonin use is not usually included among the drug-induced causes of hyperprolactinemia[11].

Based on the above, we believe that since the effect of melatonin on prolactin is tangible, the laboratory finding of hyperprolactinemia could be encountered more often, given the increase in melatonin's use. This issue merits further study, because the caveat is that the available literature has honed on acute effects of melatonin on prolactin. Thus, the effects of chronic use of melatonin on prolactin are obscure. These could be assessed initially in a simple manner: In subjects with hyperprolactinemia, medical history could query on the administration of melatonin. A further step could be a prospective study in subjects that commence melatonin supplementation.

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Country/Territory of origin: Greece

ORCID number: Evangelia Venaki 0000-0001-8942-2039; Eftychia Koukkou 0000-0002-1433-3151; Ioannis Ilias 0000-0001-5718-7441.

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REFERENCES

1 **Li J**, Somers VK, Xu H, Lopez-Jimenez F, Covassin N. Trends in Use of Melatonin Supplements Among US Adults, 1999-2018. *JAMA* 2022; **327**: 483-485 [PMID: 35103775 DOI: 10.1001/jama.2021.23652]
2 **Wan M**, Begum R, Rashed AN. Trends, geographical variation and factors associated with melatonin prescribing in general practices in England: A practice-level analysis. *Br J Clin Pharmacol* 2022; **88**: 2430-2436 [PMID: 34747044 DOI: 10.1111/bcp.15136]
3 **Grigg-Damberger MM**, Ianakieva D. Poor Quality Control of Over-the-Counter Melatonin: What They Say Is Often Not What You Get. *J Clin Sleep Med* 2017; **13**: 163-165 [PMID: 28095978 DOI: 10.5664/jcsm.6434]
4 **Ferracioli-Oda E**, Qawasmi A, Bloch MH. Meta-analysis: melatonin for the treatment of primary sleep disorders. *PLoS One* 2013; **8**: e63773 [PMID: 23691095 DOI: 10.1371/journal.pone.0063773]
5 **Colares JR**, Hartmann RM, Schemitt EG, Fonseca SRB, Brasil MS, Picada JN, Dias AS, Bueno AF, Marroni CA, Marroni NP. Melatonin prevents oxidative stress, inflammatory activity, and DNA damage in cirrhotic rats. *World J Gastroenterol* 2022; **28**: 348-364 [PMID: 35110954 DOI: 10.3748/wjg.v28.i3.348]
6 **You W**, Fan XY, Lei C, Nie CC, Chen Y, Wang XL. Melatonin intervention to prevent delirium in hospitalized patients: A meta-analysis. *World J Clin Cases* 2022; **10**: 3773-3786 [PMID: 35647160 DOI: 10.12998/wjcc.v10.i12.3773]
7 **Duffy JF**, Wang W, Ronda JM, Czeisler CA. High dose melatonin increases sleep duration during nighttime and daytime sleep episodes in older adults. *J Pineal Res* 2022; **73**: e12801 [PMID: 35436355 DOI: 10.1111/jpi.12801]
8 **Forsling ML**, Wheeler MJ, Williams AJ. The effect of melatonin administration on pituitary hormone secretion in man. *Clin Endocrinol (Oxf)* 1999; **51**: 637-642 [PMID: 10594526 DOI: 10.1046/j.1365-2265.1999.00820.x]
9 **Kostoglou-Athanassiou I**, Treacher DF, Wheeler MJ, Forsling ML. Melatonin administration and pituitary hormone

secretion. *Clin Endocrinol (Oxf)* 1998; **48**: 31-37 [PMID: 9509065 DOI: 10.1046/j.1365-2265.1998.00341.x]

10 **Lau CS**, Aw TC. A Current Approach to Hyperprolactinemia. *Int Arch Endocrinol Clin Res* 2019; **5**: 018 [DOI: 10.23937/2572-407X.1510018]

11 **Vilar L**, Vilar CF, Lyra R, Freitas MDC. Pitfalls in the Diagnostic Evaluation of Hyperprolactinemia. *Neuroendocrinology* 2019; **109**: 7-19 [PMID: 30889571 DOI: 10.1159/000499694]



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ORIGINAL ARTICLE

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REVIEW

Physiopathological mechanisms related to inflammation in obesity and type 2 diabetes mellitus

Ioannis G Lempesis, Vasiliki E Georgakopoulou

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Ioannis G Lempesis, Vasiliki E Georgakopoulou, Department of Infectious Diseases-COVID-19 Unit, Laiko General Hospital, Athens 11527, Greece

Corresponding author: Vasiliki E Georgakopoulou, Doctor, MSc, Doctor, Department of Infectious Diseases-COVID-19 Unit, Laiko General Hospital, 17 Agiou Thoma Street, Athens 11527, Greece. vaso_georgakopoulou@hotmail.com

Abstract

Overweight, obesity, and type 2 diabetes mellitus pose global health problems that are ever-increasing. A chronic low-grade inflammatory status and the presence of various pro-inflammatory markers either in circulation or within dysfunctional metabolic tissues are well established. The presence of these factors can, to some extent, predict disease development and progression. A central role is played by the presence of dysfunctional adipose tissue, liver dysfunction, and skeletal muscle dysfunction, which collectively contribute to the increased circulatory levels of proinflammatory factors. Weight loss and classical metabolic interventions achieve a decrease in many of these factors' circulating levels, implying that a better understanding of the processes or even the modulation of inflammation may alleviate these diseases. This review suggests that inflammation plays a significant role in the development and progression of these conditions and that measuring inflammatory markers may be useful for assessing disease risk and development of future treatment methods.

Key Words: Adipose tissue; Biomarkers; Diabetes; Inflammation; Obesity pathophysiology

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Core Tip: A significant amount of literature indicates the relationship between increased inflammatory markers and overweight, obesity, and type 2 diabetes mellitus. Even though the role of inflammation in the development and progression of these conditions is uncertain, the potential use of inflammatory markers as diagnostic and prognostic tools is under vigorous investigation. Weight loss and lifestyle interventions result on reduction of inflammatory markers in individuals with overweight and obesity and/or type 2 diabetes mellitus.

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INTRODUCTION

Overweight and obesity represent a significant, ever-increasing global public health challenge[1-4]. Obesity is a complex multifactorial disorder linked to a high risk of developing type 2 diabetes mellitus (T2DM), cardiometabolic diseases and most recently coronavirus disease 2019 (COVID-19)[2,5-10]. Excess and ectopic adiposity and adipose tissue (AT) dysfunction, characterized by a state of low-grade inflammation, underline the pathophysiology of obesity and its consequences to a great extent[8,11-18]. The presence of inflammation in obesity and related metabolic diseases is well established and is proposed to be linked to insulin resistance and/or its further exacerbation, as inflammatory mediators appear to interfere with insulin signal transduction in important metabolic organs (AT, liver, and muscle)[15,19]. Inflammatory markers may be indicators of disease development, allowing us to potentially predict the transition and/or development of complications such as T2DM and cardiovascular diseases[20,21]. Consequently, by achieving an improved understanding of the changes in metabolic and inflammatory processes in these various tissues and organs, and unveiling their properties, we could achieve a better understanding of the pathophysiology of obesity-related complications, including T2DM, and develop better prevention and treatment strategies[22-24]. In this mini-review, we will present evidence of obesity and T2DM-related inflammation, explore the underlying mechanisms in selected tissues and organs, and present potential therapeutic options based on the current literature.

CIRCULATING INFLAMMATORY MARKERS

Systemic inflammation in overweight and obesity

Excess adiposity is related to modestly raised levels of many circulating cytokines and inflammatory factors in both mice and humans; hence, obesity is usually defined as a condition of persistent low-grade systemic inflammation[15,16,25]. Evidence suggests that many of these factors are produced in AT, collectively referred to as adipokines, including hormones (leptin, adiponectin), hormone-cleavage enzymes like dipeptidyl peptidase 4, components or factors regulating the clotting cascade like plasminogen activator inhibitor-1 (PAI-1), and chemoattraction/pro-inflammatory factors including interleukins 1, 6, and 8 (IL-1, -6, -8), tumour necrosis factor alpha (TNF-α), and monocyte chemoattractant protein-1 (MCP-1)[11,22,26-30]. Adipokine expression and/or secretion is altered in states of AT dysfunction and may contribute to obesity-associated diseases[11,26]. Leptin circulating concentrations are elevated in individuals with obesity, and its concentrations are generally strongly and positively correlated with white AT mass[31,32]. At the same time, hypoadiponectinemia is another hallmark of obesity, suggesting a loss of its positive insulin-sensitizing and anti-inflammatory properties[33-35]. In a recent meta-analysis of 60 studies with 45,210 participants, positive correlations with C-reactive protein (CRP), IL-6, and TNF-α were observed for leptin but not for adiponectin, implying an important association between AT hormonal function and inflammatory biomarkers with potentially pathophysiological implications[36]. Moreover, acute-phase proteins, including CRP and serum amyloid A, and white blood cell (WBC) count, are also elevated in obesity and related metabolic diseases[21,29,37-40].

Several genome-wide association studies (GWAS) have been conducted to explore the link between obesity and various conditions, as well as the potential cause-and-effect relationship[41]. These studies have identified over 300 single-nucleotide polymorphisms that are associated with different measures of obesity, such as body mass index (BMI), waist-hip ratio, and other indicators of adiposity[41]. In a large-scale genome-wide association study involving a total of over 40000 individuals of European descent, genetic variants associated with higher BMI were strongly associated with higher high-sensitive CRP levels, indicating a causal relationship between adiposity and inflammation, however the opposite was not recorded[42].

Notably, pro-inflammatory markers were also strongly associated with insulin resistance in most individuals, regardless of the degree of adiposity, implicating either a role or at least a relationship between these molecules and the transition to more insulin-resistant states like T2DM[21,43,44].

Inflammation in T2DM and related cardiovascular complications

Subclinical chronic inflammation appears to be a distinct contributor to the development of T2DM[21,45]. Independent of the initial degree of insulin resistance and obesity, elevated levels of several inflam-

matory biomarkers at baseline in different human populations are predictive of T2DM occurrence[21,45]. Elevated levels of IL-6 and CRP are significantly associated with an increased risk of T2DM[46-48]. An elevated WBC count is also associated with a worsening of insulin sensitivity and predicts the development of T2DM[38]. Increased circulating concentrations of pro-inflammatory cytokines IL-1β, IL-18, TNF-α, (apart from IL-6 and CRP) and low levels of adiponectin are strongly associated with T2DM[49]. Among the markers of blunted fibrinolysis, increased PAI-1 appeared to predict T2DM development independent of insulin resistance and other known risk factors for diabetes[50]. Furthermore, biomarkers indicative of inflammation and endothelial dysfunction, including intercellular adhesion molecule 1 and E-selectin, were positively associated with the incidence of T2DM [51]. Based on these observations, it could also be claimed that these changes may be associated with the various cardiovascular complications often related to T2DM and obesity[52,53].

Finally, a plethora of GWAS has been conducted more recently regarding the association and causality between T2DM and inflammatory biomarkers[54]. Of these IL-1 and IL-6 pathways appeared to be positively associated, however evidence remains elusive[55,56].

PATHOPHYSIOLOGICAL BACKGROUND

As mentioned already, inflammation appears to be linked to insulin resistance and/or its worsening in obesity since it was shown to interfere with insulin signal transduction in critical metabolic organs (AT, liver, and muscle) and potentially contribute to the development of T2DM[15,19,48]. In this section, we will present potential mechanisms driving obesity-related inflammation, primarily in the AT, and the implications of circulatory inflammatory factors on various metabolic and regulatory organs. A summary can be found in Figure 1.

Adipose tissue dysfunction

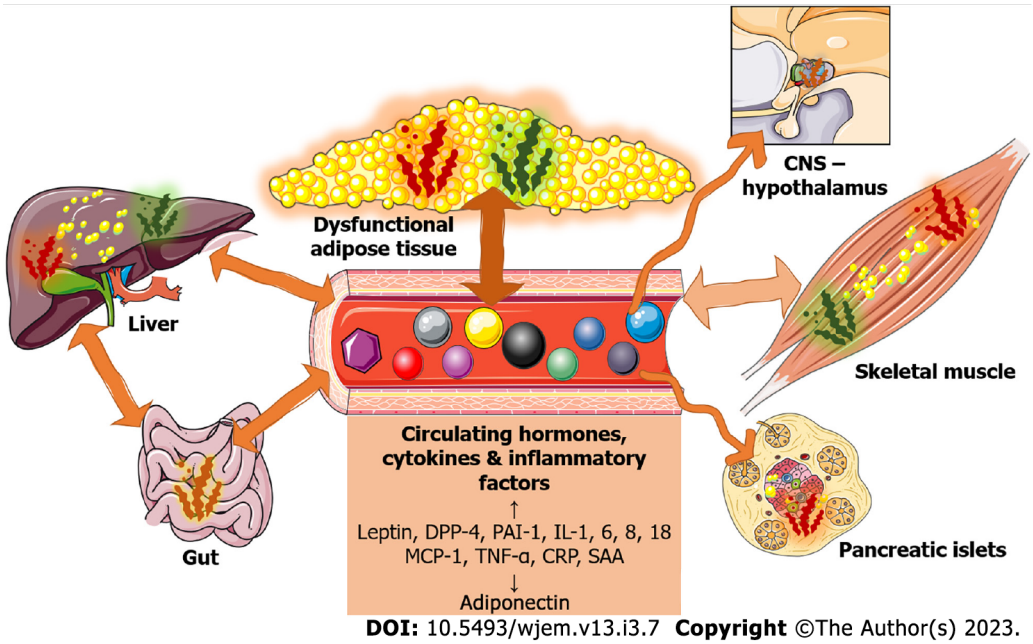
Excess adiposity, AT dysfunction (characterized by a state of low-grade inflammation), body fat distribution, and ectopic fat deposition, particularly visceral fat deposition, are all central figures in the pathophysiology of obesity and its complications[8,11,14].

Dysfunctional AT is distinguished by adipocyte hypertrophy, which is associated with chronic low-grade inflammation, pro-inflammatory immune cell infiltration, adipokine dysregulation, hormonal resistance, blunted metabolism, reactive oxygen species production, endoplasmic reticulum stress, mitochondrial dysfunction, and altered oxygenation, all of which contribute to ectopic fat accumulation and related complications[13,15,57,58]. The location of lipid storage is a key factor in determining an individual's overall health, as obesity-related complications such as hypertension and the risk of T2DM relate to abdominal (upper body) fat accumulation[14,59-63]. In contrast, fat accumulation in the lower body (gluteofemoral AT) is linked to a lower risk of cardiometabolic disease[59,64-67]. Lower body cell composition, including immune cells, is thought to be primarily associated with enhanced anti-inflammatory properties[17,18]. In accordance with that theory, IL-6 release (as determined by an arteriovenous difference technique model) was much lower in femoral adipose tissue than in matched abdominal tissue[60].

Obesity-related lipid accumulation in non-adipose tissues has significant metabolic effects since it is linked to insulin resistance and, potentially, through molecular mimicry, lipid moieties may trigger inflammatory pathways[11,22,68]. Furthermore, hypertrophic adipocytes are shown to possess a pro-inflammatory phenotype, which may exacerbate insulin resistance, resulting in a vicious cycle[69,70]. Adipocyte and AT inflammation, on the other hand, appears to be required for healthy AT growth and remodeling[71]. That observation implies that inflammation is more than just a harmful process, maybe contributing to AT adaptation to stressors, including excess calorie intake. It is worth noting that drugs used to treat T2DM may reduce inflammation by lowering hyperglycemia. However, these medicines' anti-inflammatory effects are inconsistent, and it is unclear if their favorable metabolic effects are mediated by regulation of chronic low-grade inflammation[72]. Finally, in addition to white AT inflammation in obesity, it appears that brown AT inflammation also exists, at least in animal models of obesity, implicating that dysregulation of this tissue aggregates the obesity-related inflammatory status [73].

Liver

Liver dysfunction linked with obesity, which encompasses the Metabolic Associated Fatty Liver Disease (MAFLD) spectrum, is characterized by complex pathophysiological processes that are currently under vigorous investigation and involve several pathways[74]. It has been postulated that an inability to sufficiently enhance subcutaneous AT triglyceride storage capacity in response to increased caloric consumption and body weight reroutes lipids towards other organs, such as the skeletal muscle and the liver, resulting in ectopic lipid accumulation and lipotoxicity at the cellular level, which leads to insulin resistance (IR) and inflammatory responses in these organs[75-79]. Interestingly, fat molecules appear to serve as ligands for substantial inflammatory signaling pathways in Kupffer cells in the liver and AT macrophages *via* the toll-like receptor 2 and 4 (TLR2/TLR4) signaling cascade[80]. Numerous previous



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Figure 1 Potential mechanisms driving obesity-related inflammation, primarily in adipose tissue and other metabolic organs and implications of circulatory inflammatory factors on various metabolic and regulatory organs. CNS: Central nervous system; CRP: C-reactive protein; DPP-4: Dipeptidyl peptidase 4; IL: Interleukins; MCP-1: Monocyte chemoattractant protein-1; SAA: Serum amyloid A; TNF-α: Tumour necrosis factor alpha. Parts of the figure were drawn by using pictures from Servier Medical Art (available from: smart.servier.com).

studies have shown that pro-inflammatory cytokines, specifically TNF- and IL-6, play an important role in the development and progression of NASH[81,82]. TNF- and IL-6 Levels are elevated in the livers and blood of NASH patients, but blocking these mediators improved MAFLD in animal models[81,83].

Muscle

Several pro-inflammatory cytokines have been reported to be overexpressed apart from AT and the liver, also in the skeletal muscle of individuals with obesity and insulin resistance as well as in animal models[84,85]. Obesity progression increases inflammation in skeletal muscle in two ways: Indirectly through AT inflammation and adipocytokines dysregulation, which affect skeletal muscle function and may also augment IR, and directly through ectopic lipid deposition within the skeletal muscle, which initiates several pro-inflammatory pathways[81,86,87]. Myocytes stimulate the production of several hormones and cytokines, collectively called myokines, including IL-6 and IL-15, as well as other molecules like fibroblast growth factor 21 (FGF21) and irisin[75,82]. All these molecules can regulate potential inflammatory processes, and the imbalance of their production in IR, obesity, and T2DM could further aggregate this overall inflammatory status[88]. Sarcopenic obesity, which is more common in older patients, may also cause an increase in unfavorable pro-inflammatory status and impair insulin sensitivity *via* a loss of favorable myokines[9]. Finally, as in the AT and other organs, immune cell infiltration with pro-inflammatory activation in skeletal muscle has been observed, resulting in the release of pro-inflammatory markers such as IL-1, IL-6, and TNF-α[89,90].

Other important tissues and organs dysfunction/inflammation

Evidence exists that several other tissues and organs in obesity, T2DM, and insulin resistance states are affected by or involved in systemic inflammation. For example, it is well established that macrophage infiltration is associated with islet inflammation and cell abnormalities in T2DM and obesity[91-93]. Furthermore, the western diet and cultural habits may be part of a vicious cycle that promotes oxidative stress and inflammation in the gut and even the brain[94]. Obesity-related inflammation is enhanced by diminished mucosal barriers and intestinal immune homeostasis[95]. These findings could be attributed to effects on the gut microbiome[96]; the importance of diet on organ-specific and systemic inflammation is apparent in diet-induced models of obesity in which even parts of the brain, in particular the hypothalamic arcuate nucleus, have been affected *via* infiltration of macrophages and increased expression of pro-inflammatory markers[16,97-99]. Finally, even non-metabolic or metabolic regulatory organs seem to be affected, as obesity appears to induce ovarian and kidney inflammation, respiratory hyperresponsiveness, and various hematological consequences[100-103].

IMPACT OF CLASSICAL METABOLIC TREATMENTS ON INFLAMMATION AND THE THERAPEUTIC POTENTIAL OF INFLAMMATORY MODIFICATIONS IN METABOLIC DISEASES

Strategies to tackle obesity, diabetes, and related cardiometabolic diseases include a variety of combinations, including lifestyle changes with dietary and exercise options, anti-obesity, anti-diabetic, and anti-hyperlipidemic medications, bariatric or metabolic surgery, and potentially the use of drugs with anti-inflammatory properties[72,104-107].

Current medicinal therapies for T2DM act in a multitude of ways to improve glycemic control, but they can also be beneficial for the treatment of obesity and related cardiometabolic diseases[23,24,72,107]. Many of these medications, for instance, metformin, may possess anti-inflammatory properties that can be exerted indirectly *via* metabolic control of hyperglycemia and hyperlipidemia and weight loss or by directly impacting the immune system and inflammatory responses[72,107]. Weight loss per se and therapeutic interventions that achieve it, including anti-diabetic and anti-hyperlipidemic medication use, resulted in reduced circulating concentrations of IL-6, IL-8, CRP, and MCP-1 and increased adiponectin concentrations[39,40,108-110]. A recent meta-analysis of the effect of intermittent fasting dietary patterns on plasma concentrations of inflammatory biomarkers found a decrease in CRP in individuals with overweight or obesity, but no changes in IL-6 or TNF-α[111]. A meta-analysis of 116 studies[112], however, found that serum concentrations of CRP, IL-6, and TNF- were significantly lower after bariatric surgery.

Targeting inflammation or inflammatory pathways in general has emerged as a viable alternative to traditional metabolic therapeutic options[72,107]. Anti-TNF therapy has produced contentious results in the treatment of T2DM in humans[72,113]. In animal models of IR and T2DM, inhibition combining anti-TNF and IL-1 was shown to be more effective[107,114]. Favorable effects were recorded with IL-1 blockage alone in a human study[115]. Moreover, salsalate, a prodrug of salicylate, diacerein, an anti-arthritis medication, and hydroxychloroquine, usually used for the treatment of autoimmune diseases, appeared to be beneficial; however, long-term safety profiles for these metabolic diseases are still to be elucidated[72]. Finally, the option of directly altering the pro- or anti-inflammatory activation and the balance of the immune cells within the AT arises as a potential therapeutic option[107].

CONCLUSION

In this brief review, we have demonstrated that inflammatory biomarkers reflecting underlying processes and pathway activations are present in obesity and type 2 diabetes mellitus. The impact of obesity and T2DM on inflammatory pathways appears to be linked to disease progression. Achieving a better understanding of the connection and causality between these factors and the disease risk and progression could give us the opportunity to potentially predict, follow up on, and modify their risk. Further studies are warranted to better understand the underlying pathophysiology and the use of predictive biomarkers in everyday clinical practice. It is necessary to conduct *in vivo* physiological studies to investigate the sequence of events underlying pathophysiological events in various metabolic and regulatory tissues, such as adipose tissue. Such studies can help elucidate whether inflammation precedes metabolic derangements or is mainly a result of perturbations caused by increased adiposity. Furthermore, it is essential to conduct randomized clinical trials that precisely target inflammatory pathways in diverse populations to either confirm or refute these findings.

FOOTNOTES

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ORCID number: Vasiliki E Georgakopoulou 0000-0003-0772-811X.

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REFERENCES

1 **GBD 2015 Obesity Collaborators**, Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Lee A, Marczak L, Mokdad AH, Moradi-Lakeh M, Naghavi M, Salama JS, Vos T, Abate KH, Abbafati C, Ahmed MB, Al-Aly Z, Alkerwi A, Al-Raddadi R, Amare AT, Amberbir A, Amegah AK, Amini E, Amrock SM, Anjana RM, Ärnlöv J, Asayesh H, Banerjee A, Barac A, Baye E, Bennett DA, Beyene AS, Biadgilign S, Biryukov S, Bjertness E, Boneya DJ, Campos-Nonato I, Carrero JJ, Cecilio P, Cercy K, Ciobanu LG, Cornaby L, Damtew SA, Dandona L, Dandona R, Dharmaratne SD, Duncan BB, Eshrati B, Esteghamati A, Feigin VL, Fernandes JC, Fürst T, Gebrehiwot TT, Gold A, Gona PN, Goto A, Habtewold TD, Hadush KT, Hafezi-Nejad N, Hay SI, Horino M, Islami F, Kamal R, Kasaeian A, Katikireddi SV, Kengne AP, Kesavachandran CN, Khader YS, Khang YH, Khubchandani J, Kim D, Kim YJ, Kinfu Y, Kosen S, Ku T, Defo BK, Kumar GA, Larson HJ, Leinsalu M, Liang X, Lim SS, Liu P, Lopez AD, Lozano R, Majeed A, Malekzadeh R, Malta DC, Mazidi M, McAlinden C, McGarvey ST, Mengistu DT, Mensah GA, Mensink GBM, Mezgebe HB, Mirrakhimov EM, Mueller UO, Noubiap JJ, Obermeyer CM, Ogbo FA, Owolabi MO, Patton GC, Pourmalek F, Qorbani M, Rafay A, Rai RK, Ranabhat CL, Reinig N, Safiri S, Salomon JA, Sanabria JR, Santos IS, Sartorius B, Sawhney M, Schmidhuber J, Schutte AE, Schmidt MI, Sepanlou SG, Shamsizadeh M, Sheikhbahaei S, Shin MJ, Shiri R, Shiue I, Roba HS, Silva DAS, Silverberg JI, Singh JA, Stranges S, Swaminathan S, Tabarés-Seisdedos R, Tadese F, Tedla BA, Tegegne BS, Terkawi AS, Thakur JS, Tonelli M, Topor-Madry R, Tyrovolas S, Ukwaja KN, Uthman OA, Vaezghasemi M, Vasankari T, Vlassov VV, Vollset SE, Weiderpass E, Werdecker A, Wesana J, Westerman R, Yano Y, Yonemoto N, Yonga G, Zaidi Z, Zenebe ZM, Zipkin B, Murray CJL. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *N Engl J Med* 2017; **377**: 13-27 [PMID: 28604169 DOI: 10.1056/NEJMoa1614362]

2 **Heymsfield SB**, Wadden TA. Mechanisms, Pathophysiology, and Management of Obesity. *N Engl J Med* 2017; **376**: 254-266 [PMID: 28099824 DOI: 10.1056/NEJMra1514009]

3 **González-Muniesa P**, Mártinez-González MA, Hu FB, Després JP, Matsuzawa Y, Loos RJF, Moreno LA, Bray GA, Martinez JA. Obesity. *Nat Rev Dis Primers* 2017; **3**: 17034 [PMID: 28617414 DOI: 10.1038/nrdp.2017.34]

4 **NCD Risk Factor Collaboration (NCD-RisC)**. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. *Lancet* 2016; **387**: 1377-1396 [PMID: 27115820 DOI: 10.1016/S0140-6736(16)30054-X]

5 **Zheng Y**, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol* 2018; **14**: 88-98 [PMID: 29219149 DOI: 10.1038/nrendo.2017.151]

6 **Bray GA**, Heisel WE, Afshin A, Jensen MD, Dietz WH, Long M, Kushner RF, Daniels SR, Wadden TA, Tsai AG, Hu FB, Jakicic JM, Ryan DH, Wolfe BM, Inge TH. The Science of Obesity Management: An Endocrine Society Scientific Statement. *Endocr Rev* 2018; **39**: 79-132 [PMID: 29518206 DOI: 10.1210/er.2017-00253]

7 **Martin SD**, McGee SL. Metabolic reprogramming in type 2 diabetes and the development of breast cancer. *J Endocrinol* 2018; **237**: R35-R46 [PMID: 29487204 DOI: 10.1530/JOE-18-0037]

8 **Frühbeck G**, Busetto L, Dicker D, Yumuk V, Goossens GH, Hebebrand J, Halford JGC, Farpour-Lambert NJ, Blaak EE, Woodward E, Toplak H. The ABCD of Obesity: An EASO Position Statement on a Diagnostic Term with Clinical and Scientific Implications. *Obes Facts* 2019; **12**: 131-136 [PMID: 30844811 DOI: 10.1159/000497124]

9 **Sagris M**, Kokkinidis DG, Lempesis IG, Giannopoulos S, Rallidis L, Mena-Hurtado C, Bakoyiannis C. Nutrition, dietary habits, and weight management to prevent and treat patients with peripheral artery disease. *Rev Cardiovasc Med* 2020; **21**: 565-575 [PMID: 33388001 DOI: 10.31083/j.rcm.2020.04.202]

10 **Lempesis IG**, Karlafti E, Papalexis P, Fotakopoulos G, Tarantinos K, Lekakis V, Papadakos SP, Cholongitas E, Georgakopoulou VE. COVID-19 and liver injury in individuals with obesity. *World J Gastroenterol* 2023; **29**: 908-916 [PMID: 36844135 DOI: 10.3748/wjg.v29.i6.908]

11 **Goossens GH**. The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. *Physiol Behav* 2008; **94**: 206-218 [PMID: 18037457 DOI: 10.1016/j.physbeh.2007.10.010]

12 **Blüher M**. Adipose tissue dysfunction contributes to obesity related metabolic diseases. *Best Pract Res Clin Endocrinol Metab* 2013; **27**: 163-177 [PMID: 23731879 DOI: 10.1016/j.beem.2013.02.005]

13 **Goossens GH**, Blaak EE. Adipose tissue dysfunction and impaired metabolic health in human obesity: a matter of oxygen? *Front Endocrinol (Lausanne)* 2015; **6**: 55 [PMID: 25964776 DOI: 10.3389/fendo.2015.00055]

14 **Goossens GH**. The Metabolic Phenotype in Obesity: Fat Mass, Body Fat Distribution, and Adipose Tissue Function. *Obes Facts* 2017; **10**: 207-215 [PMID: 28564650 DOI: 10.1159/000471488]

15 **Lempesis IG**, van Meijel RLJ, Manolopoulos KN, Goossens GH. Oxygenation of adipose tissue: A human perspective. *Acta Physiol (Oxf)* 2020; **228**: e13298 [PMID: 31077538 DOI: 10.1111/apha.13298]

16 **Lempesis IG**, Tsilingiris D, Liu J, Dalamaga M. Of mice and men: Considerations on adipose tissue physiology in animal models of obesity and human studies. *Metabol Open* 2022; **15**: 100208 [PMID: 36092796 DOI: 10.1016/j.metop.2022.100208]

17 **Lempesis IG**, Hoebers N, Essers Y, Jocken JWE, Rouschop KMA, Blaak EE, Manolopoulos KN, Goossens GH. Physiological Oxygen Levels Differentially Regulate Adipokine Production in Abdominal and Femoral Adipocytes from Individuals with Obesity Versus Normal Weight. *Cells* 2022; **11** [PMID: 36428961 DOI: 10.3390/cells11223532]

18 **Lempesis IG**, Hoebers N, Essers Y, Jocken JW, Blaak EE, Manolopoulos KN, Goossens GH. Distinct inflammatory signatures of upper-and lower-body adipose tissue in postmenopausal women with normal weight and obesity. *Endocrine Abstracts* 2022; **81**: EP355 [DOI: 10.1530/endoabs.81.EP355]

19 **de Luca C**, Olefsky JM. Inflammation and insulin resistance. *FEBS Lett* 2008; **582**: 97-105 [PMID: 18053812 DOI: 10.1016/j.febslet.2007.11.057]

20 **Calle MC**, Fernandez ML. Inflammation and type 2 diabetes. *Diabetes Metab* 2012; **38**: 183-191 [PMID: 22252015 DOI: 10.1016/j.diabet.2011.11.006]

21 **Esser N**, Legrand-Poels S, Piette J, Scheen AJ, Paquot N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. *Diabetes Res Clin Pract* 2014; **105**: 141-150 [PMID: 24798950 DOI: 10.1016/j.diabres.2014.04.006]

22 **Rosen ED**, Spiegelman BM. What we talk about when we talk about fat. *Cell* 2014; **156**: 20-44 [PMID: 24439368 DOI: 10.1016/j.cell.2013.12.012]

23 **Lempesis IG**, Liu J, Dalamaga M. The catcher in the gut: Tirzepatide, a dual incretin analog for the treatment of type 2 diabetes mellitus and obesity. *Metabol Open* 2022; **16**: 100220 [PMID: 36530219 DOI: 10.1016/j.metop.2022.100220]

24 **Vallianou NG**, Tsilingiris D, Kounatidis D, Lempesis IG, Karampela I, Dalamaga M. Sodiumglucose cotransporter2 inhibitors in obesity and associated cardiometabolic disorders: where do we stand? *Pol Arch Intern Med* 2022; **132** [PMID: 36094176 DOI: 10.20452/pamw.16342]

25 **Burhans MS**, Hagman DK, Kuzma JN, Schmidt KA, Kratz M. Contribution of Adipose Tissue Inflammation to the Development of Type 2 Diabetes Mellitus. *Compr Physiol* 2018; **9**: 1-58 [PMID: 30549014 DOI: 10.1002/cphy.c170040]

26 **Fasshauer M**, Blüher M. Adipokines in health and disease. *Trends Pharmacol Sci* 2015; **36**: 461-470 [PMID: 26022934 DOI: 10.1016/j.tips.2015.04.014]

27 **Lamers D**, Famulla S, Wronkowitz N, Hartwig S, Lehr S, Ouwens DM, Eckardt K, Kaufman JM, Ryden M, Müller S, Hanisch FG, Ruige J, Arner P, Sell H, Eckel J. Dipeptidyl peptidase 4 is a novel adipokine potentially linking obesity to the metabolic syndrome. *Diabetes* 2011; **60**: 1917-1925 [PMID: 21593202 DOI: 10.2337/db10-1707]

28 **Kim CS**, Park HS, Kawada T, Kim JH, Lim D, Hubbard NE, Kwon BS, Erickson KL, Yu R. Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. *Int J Obes (Lond)* 2006; **30**: 1347-1355 [PMID: 16534530 DOI: 10.1038/sj.ijo.0803259]

29 **Pickup JC**, Mattock MB, Chusney GD, Burt D. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia* 1997; **40**: 1286-1292 [PMID: 9389420 DOI: 10.1007/s001250050822]

30 **Mohamed-Ali V**, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, Klein S, Coppack SW. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J Clin Endocrinol Metab* 1997; **82**: 4196-4200 [PMID: 9398739 DOI: 10.1210/jcem.82.12.4450]

31 **Mantzoros CS**, Magkos F, Brinkoetter M, Sienkiewicz E, Dardeno TA, Kim SY, Hamnvik OP, Koniaris A. Leptin in human physiology and pathophysiology. *Am J Physiol Endocrinol Metab* 2011; **301**: E567-E584 [PMID: 21791620 DOI: 10.1152/ajpendo.00315.2011]

32 **Kalaitzopoulos DR**, Lempesis IG, Samartzis N, Kolovos G, Dedes I, Daniilidis A, Nirgianakis K, Leeners B, Goulis DG, Samartzis EP. Leptin concentrations in endometriosis: A systematic review and meta-analysis. *J Reprod Immunol* 2021; **146**: 103338 [PMID: 34126469 DOI: 10.1016/j.jri.2021.103338]

33 **Straub LG**, Scherer PE. Metabolic Messengers: Adiponectin. *Nat Metab* 2019; **1**: 334-339 [PMID: 32661510 DOI: 10.1038/s42255-019-0041-z]

34 **Santaniemi M**, Kesäniemi YA, Ukkola O. Low plasma adiponectin concentration is an indicator of the metabolic syndrome. *Eur J Endocrinol* 2006; **155**: 745-750 [PMID: 17062891 DOI: 10.1530/eje.1.02287]

35 **Achari AE**, Jain SK. Adiponectin, a Therapeutic Target for Obesity, Diabetes, and Endothelial Dysfunction. *Int J Mol Sci* 2017; **18** [PMID: 28635626 DOI: 10.3390/ijms18061321]

36 **Graßmann S**, Wirsching J, Eichelmann F, Aleksandrova K. Association Between Peripheral Adipokines and Inflammation Markers: A Systematic Review and Meta-Analysis. *Obesity (Silver Spring)* 2017; **25**: 1776-1785 [PMID: 28834421 DOI: 10.1002/oby.21945]

37 **Yudkin JS**, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* 1999; **19**: 972-978 [PMID: 10195925 DOI: 10.1161/01.atv.19.4.972]

38 **Vozarova B**, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA. High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 2002; **51**: 455-461 [PMID: 11812755 DOI: 10.2337/diabetes.51.2.455]

39 **Christiansen T**, Richelsen B, Bruun JM. Monocyte chemoattractant protein-1 is produced in isolated adipocytes, associated with adiposity and reduced after weight loss in morbid obese subjects. *Int J Obes (Lond)* 2005; **29**: 146-150 [PMID: 15520826 DOI: 10.1038/sj.ijo.0802839]

40 **Bruun JM**, Helge JW, Richelsen B, Stallknecht B. Diet and exercise reduce low-grade inflammation and macrophage infiltration in adipose tissue but not in skeletal muscle in severely obese subjects. *Am J Physiol Endocrinol Metab* 2006; **290**: E961-E967 [PMID: 16352667 DOI: 10.1152/ajpendo.00506.2005]

41 **Goodarzi MO**. Genetics of obesity: what genetic association studies have taught us about the biology of obesity and its complications. *Lancet Diabetes Endocrinol* 2018; **6**: 223-236 [PMID: 28919064 DOI: 10.1016/S2213-8587(17)30200-0]

42 **Welsh P**, Polisecki E, Robertson M, Jahn S, Buckley BM, de Craen AJ, Ford I, Jukema JW, Macfarlane PW, Packard CJ, Stott DJ, Westendorp RG, Shepherd J, Hingorani AD, Smith GD, Schaefer E, Sattar N. Unraveling the directional link between adiposity and inflammation: a bidirectional Mendelian randomization approach. *J Clin Endocrinol Metab* 2010; **95**: 93-99 [PMID: 19906786 DOI: 10.1210/jc.2009-1064]

43 **Natali A**, Toschi E, Baldeweg S, Ciociaro D, Favilla S, Saccà L, Ferrannini E. Clustering of insulin resistance with vascular dysfunction and low-grade inflammation in type 2 diabetes. *Diabetes* 2006; **55**: 1133-1140 [PMID: 16567539 DOI: 10.2337/diabetes.55.04.06.db05-1076]

44 **Phillips CM**, Perry IJ. Does inflammation determine metabolic health status in obese and nonobese adults? *J Clin Endocrinol Metab* 2013; **98**: E1610-E1619 [PMID: 23979951 DOI: 10.1210/jc.2013-2038]

45 **Dorcely B**, Katz K, Jagannathan R, Chiang SS, Oluwadare B, Goldberg IJ, Bergman M. Novel biomarkers for prediabetes, diabetes, and associated complications. *Diabetes Metab Syndr Obes* 2017; **10**: 345-361 [PMID: 28860833 DOI: 10.2147/DMSO.S100074]

46 **Wang X**, Bao W, Liu J, Ouyang YY, Wang D, Rong S, Xiao X, Shan ZL, Zhang Y, Yao P, Liu LG. Inflammatory

markers and risk of type 2 diabetes: a systematic review and meta-analysis. *Diabetes Care* 2013; **36**: 166-175 [PMID: 23264288 DOI: 10.2337/dc12-0702]

47 **Pradhan AD**, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 2001; **286**: 327-334 [PMID: 11466099 DOI: 10.1001/jama.286.3.327]

48 **Adabimohazab R**, Garfinkel A, Milam EC, Frosch O, Mangone A, Convit A. Does Inflammation Mediate the Association Between Obesity and Insulin Resistance? *Inflammation* 2016; **39**: 994-1003 [PMID: 26956471 DOI: 10.1007/s10753-016-0329-z]

49 **Liu C**, Feng X, Li Q, Wang Y, Hua M. Adiponectin, TNF-α and inflammatory cytokines and risk of type 2 diabetes: A systematic review and meta-analysis. *Cytokine* 2016; **86**: 100-109 [PMID: 27498215 DOI: 10.1016/j.cyto.2016.06.028]

50 **Festa A**, D'Agostino R Jr, Tracy RP, Haffner SM; Insulin Resistance Atherosclerosis Study. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes* 2002; **51**: 1131-1137 [PMID: 11916936 DOI: 10.2337/diabetes.51.4.1131]

51 **Odegaard AO**, Jacobs DR Jr, Sanchez OA, Goff DC Jr, Reiner AP, Gross MD. Oxidative stress, inflammation, endothelial dysfunction and incidence of type 2 diabetes. *Cardiovasc Diabetol* 2016; **15**: 51 [PMID: 27013319 DOI: 10.1186/s12933-016-0369-6]

52 **Battineni G**, Sagaro GG, Chintalapudi N, Amenta F, Tomassoni D, Tayebati SK. Impact of Obesity-Induced Inflammation on Cardiovascular Diseases (CVD). *Int J Mol Sci* 2021; **22** [PMID: 33946540 DOI: 10.3390/ijms22094798]

53 **Qu D**, Liu J, Lau CW, Huang Y. IL-6 in diabetes and cardiovascular complications. *Br J Pharmacol* 2014; **171**: 3595-3603 [PMID: 24697653 DOI: 10.1111/bph.12713]

54 **Yuan S**, Merino J, Larsson SC. Causal factors underlying diabetes risk informed by Mendelian randomisation analysis: evidence, opportunities and challenges. *Diabetologia* 2023; **66**: 800-812 [PMID: 36786839 DOI: 10.1007/s00125-023-05879-7]

55 **Yuan S**, Larsson SC. An atlas on risk factors for type 2 diabetes: a wide-angled Mendelian randomisation study. *Diabetologia* 2020; **63**: 2359-2371 [PMID: 32895727 DOI: 10.1007/s00125-020-05253-x]

56 **Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium**, Swerdlow DI, Holmes MV, Kuchenbaecker KB, Engmann JE, Shah T, Sofat R, Guo Y, Chung C, Peasey A, Pfister R, Mooijaart SP, Ireland HA, Leusink M, Langenberg C, Li KW, Palmen J, Howard P, Cooper JA, Drenos F, Hardy J, Nalls MA, Li YR, Lowe G, Stewart M, Bielinski SJ, Peto J, Timpson NJ, Gallacher J, Dunlop M, Houlston R, Tomlinson I, Tzoulaki I, Luan J, Boer JM, Forouhi NG, Onland-Moret NC, van der Schouw YT, Schnabel RB, Hubacek JA, Kubinova R, Baceviciene M, Tamosiunas A, Pajak A, Topor-Madry R, Malyutina S, Baldassarre D, Sennblad B, Tremoli E, de Faire U, Ferrucci L, Bandenelli S, Tanaka T, Meschia JF, Singleton A, Navis G, Mateo Leach I, Bakker SJ, Gansevoort RT, Ford I, Epstein SE, Burnett MS, Devaney JM, Jukema JW, Westendorp RG, Jan de Borst G, van der Graaf Y, de Jong PA, Mailand-van der Zee AH, Klungel OH, de Boer A, Doevendans PA, Stephens JW, Eaton CB, Robinson JG, Manson JE, Fowkes FG, Frayling TM, Price JF, Whincup PH, Morris RW, Lawlor DA, Smith GD, Ben-Shlomo Y, Redline S, Lange LA, Kumari M, Wareham NJ, Verschuren WM, Benjamin EJ, Whittaker JC, Hamsten A, Dudbridge F, Delaney JA, Wong A, Kuh D, Hardy R, Castillo BA, Connolly JJ, van der Harst P, Brunner EJ, Marmot MG, Wassel CL, Humphries SE, Talmud PJ, Kivimaki M, Asselbergs FW, Voevoda M, Bobak M, Pikhart H, Wilson JG, Hakonarson H, Reiner AP, Keating BJ, Sattar N, Hingorani AD, Casas JP. The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. *Lancet* 2012; **379**: 1214-1224 [PMID: 22421340 DOI: 10.1016/S0140-6736(12)60110-X]

57 **Shulman GI**. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. *N Engl J Med* 2014; **371**: 1131-1141 [PMID: 25229917 DOI: 10.1056/NEJMra1011035]

58 **Frayn KN**, Karpe F. Regulation of human subcutaneous adipose tissue blood flow. *Int J Obes (Lond)* 2014; **38**: 1019-1026 [PMID: 24166067 DOI: 10.1038/ijo.2013.200]

59 **Manolopoulos KN**, Karpe F, Frayn KN. Gluteofemoral body fat as a determinant of metabolic health. *Int J Obes (Lond)* 2010; **34**: 949-959 [PMID: 20065965 DOI: 10.1038/ijo.2009.286]

60 **Karpe F**, Pinnick KE. Biology of upper-body and lower-body adipose tissue--link to whole-body phenotypes. *Nat Rev Endocrinol* 2015; **11**: 90-100 [PMID: 25365922 DOI: 10.1038/nrendo.2014.185]

61 **Canoy D**, Luben R, Welch A, Bingham S, Wareham N, Day N, Khaw KT. Fat distribution, body mass index and blood pressure in 22,090 men and women in the Norfolk cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Norfolk) study. *J Hypertens* 2004; **22**: 2067-2074 [PMID: 15480089 DOI: 10.1097/00004872-200411000-00007]

62 **Grundy SM**, Adams-Huet B, Vega GL. Variable contributions of fat content and distribution to metabolic syndrome risk factors. *Metab Syndr Relat Disord* 2008; **6**: 281-288 [PMID: 18759660 DOI: 10.1089/met.2008.0026]

63 **Meisinger C**, Döring A, Thorand B, Heier M, Löwel H. Body fat distribution and risk of type 2 diabetes in the general population: are there differences between men and women? The MONICA/KORA Augsburg cohort study. *Am J Clin Nutr* 2006; **84**: 483-489 [PMID: 16960160 DOI: 10.1093/ajcn/84.3.483]

64 **Seidell JC**, Pérusse L, Després JP, Bouchard C. Waist and hip circumferences have independent and opposite effects on cardiovascular disease risk factors: the Quebec Family Study. *Am J Clin Nutr* 2001; **74**: 315-321 [PMID: 11522554 DOI: 10.1093/ajcn/74.3.315]

65 **Terry RB**, Stefanick ML, Haskell WL, Wood PD. Contributions of regional adipose tissue depots to plasma lipoprotein concentrations in overweight men and women: possible protective effects of thigh fat. *Metabolism* 1991; **40**: 733-740 [PMID: 1870428 DOI: 10.1016/0026-0495(91)90093-c]

66 **Williams MJ**, Hunter GR, Kekes-Szabo T, Snyder S, Treuth MS. Regional fat distribution in women and risk of cardiovascular disease. *Am J Clin Nutr* 1997; **65**: 855-860 [PMID: 9062540 DOI: 10.1093/ajcn/65.3.855]

67 **Yim JE**, Heshka S, Albu JB, Heymsfield S, Gallagher D. Femoral-gluteal subcutaneous and intermuscular adipose tissues have independent and opposing relationships with CVD risk. *J Appl Physiol (1985)* 2008; **104**: 700-707 [PMID: 18079271 DOI: 10.1152/japplphysiol.01035.2007]

68 **Haczeyni F**, Bell-Anderson KS, Farrell GC. Causes and mechanisms of adipocyte enlargement and adipose expansion. *Obes Rev* 2018; **19**: 406-420 [PMID: 29243339 DOI: 10.1111/obr.12646]

69 **Skurk T**, Alberti-Huber C, Herder C, Hauner H. Relationship between adipocyte size and adipokine expression and secretion. *J Clin Endocrinol Metab* 2007; **92**: 1023-1033 [PMID: 17164304 DOI: 10.1210/jc.2006-1055]

70 **Sun S**, Ji Y, Kersten S, Qi L. Mechanisms of inflammatory responses in obese adipose tissue. *Annu Rev Nutr* 2012; **32**: 261-286 [PMID: 22404118 DOI: 10.1146/annurev-nutr-071811-150623]

71 **Wernstedt Asterholm I**, Tao C, Morley TS, Wang QA, Delgado-Lopez F, Wang ZV, Scherer PE. Adipocyte inflammation is essential for healthy adipose tissue expansion and remodeling. *Cell Metab* 2014; **20**: 103-118 [PMID: 24930973 DOI: 10.1016/j.cmet.2014.05.005]

72 **Pollack RM**, Donath MY, LeRoith D, Leibowitz G. Anti-inflammatory Agents in the Treatment of Diabetes and Its Vascular Complications. *Diabetes Care* 2016; **39** Suppl 2: S244-S252 [PMID: 27440839 DOI: 10.2337/dcS15-3015]

73 **Villarroya F**, Cereijo R, Gavaldà-Navarro A, Villarroya J, Giralt M. Inflammation of brown/beige adipose tissues in obesity and metabolic disease. *J Intern Med* 2018; **284**: 492-504 [PMID: 29923291 DOI: 10.1111/joim.12803]

74 **Sarwar R**, Pierce N, Koppe S. Obesity and nonalcoholic fatty liver disease: current perspectives. *Diabetes Metab Syndr Obes* 2018; **11**: 533-542 [PMID: 30288073 DOI: 10.2147/DMSO.S146339]

75 **Beals JW**, Smith GI, Shankaran M, Fuchs A, Schweitzer GG, Yoshino J, Field T, Matthews M, Nyangau E, Morozov D, Mittendorfer B, Hellerstein MK, Klein S. Increased Adipose Tissue Fibrogenesis, Not Impaired Expandability, Is Associated With Nonalcoholic Fatty Liver Disease. *Hepatology* 2021; **74**: 1287-1299 [PMID: 33743554 DOI: 10.1002/hep.31822]

76 **Ter Horst KW**, Gilijamse PW, Versteeg RI, Ackermans MT, Nederveen AJ, la Fleur SE, Romijn JA, Nieuwdorp M, Zhang D, Samuel VT, Vatner DF, Petersen KF, Shulman GI, Serlie MJ. Hepatic Diacylglycerol-Associated Protein Kinase Cε Translocation Links Hepatic Steatosis to Hepatic Insulin Resistance in Humans. *Cell Rep* 2017; **19**: 1997-2004 [PMID: 28591572 DOI: 10.1016/j.celrep.2017.05.035]

77 **Korenblat KM**, Fabbrini E, Mohammed BS, Klein S. Liver, muscle, and adipose tissue insulin action is directly related to intrahepatic triglyceride content in obese subjects. *Gastroenterology* 2008; **134**: 1369-1375 [PMID: 18355813 DOI: 10.1053/j.gastro.2008.01.075]

78 **Sanyal AJ**, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 2001; **120**: 1183-1192 [PMID: 11266382 DOI: 10.1053/gast.2001.23256]

79 **Sharpton SR**, Schnabl B, Knight R, Loomba R. Current Concepts, Opportunities, and Challenges of Gut Microbiome-Based Personalized Medicine in Nonalcoholic Fatty Liver Disease. *Cell Metab* 2021; **33**: 21-32 [PMID: 33296678 DOI: 10.1016/j.cmet.2020.11.010]

80 **Schenk S**, Saberi M, Olefsky JM. Insulin sensitivity: modulation by nutrients and inflammation. *J Clin Invest* 2008; **118**: 2992-3002 [PMID: 18769626 DOI: 10.1172/JCI34260]

81 **Luo Y**, Lin H. Inflammation initiates a vicious cycle between obesity and nonalcoholic fatty liver disease. *Immun Inflamm Dis* 2021; **9**: 59-73 [PMID: 33332766 DOI: 10.1002/iid3.391]

82 **Feldstein AE**. Novel insights into the pathophysiology of nonalcoholic fatty liver disease. *Semin Liver Dis* 2010; **30**: 391-401 [PMID: 20960378 DOI: 10.1055/s-0030-1267539]

83 **Tilg H**. The role of cytokines in non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 179-185 [PMID: 20460908 DOI: 10.1159/000282083]

84 **Gregor MF**, Hotamisligil GS. Inflammatory mechanisms in obesity. *Annu Rev Immunol* 2011; **29**: 415-445 [PMID: 21219177 DOI: 10.1146/annurev-immunol-031210-101322]

85 **Jorquera G**, Russell J, Monsalves-Álvarez M, Cruz G, Valladares-Ide D, Basualto-Alarcón C, Barrientos G, Estrada M, Llanos P. NLRP3 Inflammasome: Potential Role in Obesity Related Low-Grade Inflammation and Insulin Resistance in Skeletal Muscle. *Int J Mol Sci* 2021; **22** [PMID: 33806797 DOI: 10.3390/ijms22063254]

86 **Therkelsen KE**, Pedley A, Speliotes EK, Massaro JM, Murabito J, Hoffmann U, Fox CS. Intramuscular fat and associations with metabolic risk factors in the Framingham Heart Study. *Arterioscler Thromb Vasc Biol* 2013; **33**: 863-870 [PMID: 23349188 DOI: 10.1161/ATVBAHA.112.301009]

87 **Hotamisligil GS**. Inflammation, metaflammation and immunometabolic disorders. *Nature* 2017; **542**: 177-185 [PMID: 28179656 DOI: 10.1038/nature21363]

88 **Eckardt K**, Görgens SW, Raschke S, Eckel J. Myokines in insulin resistance and type 2 diabetes. *Diabetologia* 2014; **57**: 1087-1099 [PMID: 24676645 DOI: 10.1007/s00125-014-3224-x]

89 **Wang T**, He C. Pro-inflammatory cytokines: The link between obesity and osteoarthritis. *Cytokine Growth Factor Rev* 2018; **44**: 38-50 [PMID: 30340925 DOI: 10.1016/j.cytogfr.2018.10.002]

90 **Fink LN**, Oberbach A, Costford SR, Chan KL, Sams A, Blüher M, Klip A. Expression of anti-inflammatory macrophage genes within skeletal muscle correlates with insulin sensitivity in human obesity and type 2 diabetes. *Diabetologia* 2013; **56**: 1623-1628 [PMID: 23595247 DOI: 10.1007/s00125-013-2897-x]

91 **Eguchi K**, Nagai R. Islet inflammation in type 2 diabetes and physiology. *J Clin Invest* 2017; **127**: 14-23 [PMID: 28045399 DOI: 10.1172/JCI88877]

92 **McNelis JC**, Olefsky JM. Macrophages, immunity, and metabolic disease. *Immunity* 2014; **41**: 36-48 [PMID: 25035952 DOI: 10.1016/j.immuni.2014.05.010]

93 **Ying W**, Fu W, Lee YS, Olefsky JM. The role of macrophages in obesity-associated islet inflammation and β-cell abnormalities. *Nat Rev Endocrinol* 2020; **16**: 81-90 [PMID: 31836875 DOI: 10.1038/s41574-019-0286-3]

94 **Kopp W**. How Western Diet And Lifestyle Drive The Pandemic Of Obesity And Civilization Diseases. *Diabetes Metab Syndr Obes* 2019; **12**: 2221-2236 [PMID: 31695465 DOI: 10.2147/DMSO.S216791]

95 **Ding S**, Chi MM, Scull BP, Rigby R, Schwerbrock NM, Magness S, Jobin C, Lund PK. High-fat diet: bacteria interactions promote intestinal inflammation which precedes and correlates with obesity and insulin resistance in mouse. *PLoS One* 2010; **5**: e12191 [PMID: 20808947 DOI: 10.1371/journal.pone.0012191]

96 **Saad MJ**, Santos A, Prada PO. Linking Gut Microbiota and Inflammation to Obesity and Insulin Resistance. *Physiology (Bethesda)* 2016; **31**: 283-293 [PMID: 27252163 DOI: 10.1152/physiol.00041.2015]

97 **Thaler JP**, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, Zhao X, Sarruf DA, Izgur V, Maravilla KR, Nguyen HT, Fischer JD, Matsen ME, Wisse BE, Morton GJ, Horvath TL, Baskin DG, Tschöp MH, Schwartz MW. Obesity is associated with hypothalamic injury in rodents and humans. *J Clin Invest* 2012; **122**: 153-162 [PMID: 22201683 DOI: 10.1172/JCI59660]

98 **Araújo EP**, Torsoni MA, Velloso LA. Hypothalamic inflammation and obesity. *Vitam Horm* 2010; **82**: 129-143 [PMID: 20472136 DOI: 10.1016/S0083-6729(10)82007-2]

99 **Thaler JP**, Guyenet SJ, Dorfman MD, Wisse BE, Schwartz MW. Hypothalamic inflammation: marker or mechanism of obesity pathogenesis? *Diabetes* 2013; **62**: 2629-2634 [PMID: 23881189 DOI: 10.2337/db12-1605]

100 **Snider AP**, Wood JR. Obesity induces ovarian inflammation and reduces oocyte quality. *Reproduction* 2019; **158**: R79-R90 [PMID: 30999278 DOI: 10.1530/REP-18-0583]

101 **Navarro-Díaz M**, Serra A, López D, Granada M, Bayés B, Romero R. Obesity, inflammation, and kidney disease. *Kidney Int Suppl* 2008; S15-S18 [PMID: 19034319 DOI: 10.1038/ki.2008.518]

102 **Purdy JC**, Shatzel JJ. The hematologic consequences of obesity. *Eur J Haematol* 2021; **106**: 306-319 [PMID: 33270290 DOI: 10.1111/ejh.13560]

103 **Shore SA**. Obesity, airway hyperresponsiveness, and inflammation. *J Appl Physiol (1985)* 2010; **108**: 735-743 [PMID: 19875711 DOI: 10.1152/japplphysiol.00749.2009]

104 **Zhang C**, Liu S, Yang M. Hepatocellular Carcinoma and Obesity, Type 2 Diabetes Mellitus, Cardiovascular Disease: Causing Factors, Molecular Links, and Treatment Options. *Front Endocrinol (Lausanne)* 2021; **12**: 808526 [PMID: 35002979 DOI: 10.3389/fendo.2021.808526]

105 **Stefan N**. Causes, consequences, and treatment of metabolically unhealthy fat distribution. *Lancet Diabetes Endocrinol* 2020; **8**: 616-627 [PMID: 32559477 DOI: 10.1016/S2213-8587(20)30110-8]

106 **Romero-Gómez M**, Zelber-Sagi S, Trenell M. Treatment of NAFLD with diet, physical activity and exercise. *J Hepatol* 2017; **67**: 829-846 [PMID: 28545937 DOI: 10.1016/j.jhep.2017.05.016]

107 **AlZaim I**, Hammoud SH, Al-Koussa H, Ghazi A, Eid AH, El-Yazbi AF. Adipose Tissue Immunomodulation: A Novel Therapeutic Approach in Cardiovascular and Metabolic Diseases. *Front Cardiovasc Med* 2020; **7**: 602088 [PMID: 33282920 DOI: 10.3389/fcvm.2020.602088]

108 **Bastard JP**, Jardel C, Bruckert E, Blondy P, Capeau J, Laville M, Vidal H, Hainque B. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J Clin Endocrinol Metab* 2000; **85**: 3338-3342 [PMID: 10999830 DOI: 10.1210/jcem.85.9.6839]

109 **Haffner S**, Temprosa M, Crandall J, Fowler S, Goldberg R, Horton E, Marcovina S, Mather K, Orchard T, Ratner R, Barrett-Connor E; Diabetes Prevention Program Research Group. Intensive lifestyle intervention or metformin on inflammation and coagulation in participants with impaired glucose tolerance. *Diabetes* 2005; **54**: 1566-1572 [PMID: 15855347 DOI: 10.2337/diabetes.54.5.1566]

110 **Belalcazar LM**, Haffner SM, Lang W, Hoogeveen RC, Rushing J, Schwenke DC, Tracy RP, Pi-Sunyer FX, Kriska AM, Ballantyne CM; Look AHEAD (Action for Health in Diabetes) Research Group. Lifestyle intervention and/or statins for the reduction of C-reactive protein in type 2 diabetes: from the look AHEAD study. *Obesity (Silver Spring)* 2013; **21**: 944-950 [PMID: 23512860 DOI: 10.1002/oby.20431]

111 **Wang X**, Yang Q, Liao Q, Li M, Zhang P, Santos HO, Kord-Varkaneh H, Abshirini M. Effects of intermittent fasting diets on plasma concentrations of inflammatory biomarkers: A systematic review and meta-analysis of randomized controlled trials. *Nutrition* 2020; **79-80**: 110974 [PMID: 32947129 DOI: 10.1016/j.nut.2020.110974]

112 **Askarpour M**, Khani D, Sheikhi A, Ghaedi E, Alizadeh S. Effect of Bariatric Surgery on Serum Inflammatory Factors of Obese Patients: a Systematic Review and Meta-Analysis. *Obes Surg* 2019; **29**: 2631-2647 [PMID: 31093862 DOI: 10.1007/s11695-019-03926-0]

113 **Donath MY**, Dalmas É, Sauter NS, Böni-Schnetzler M. Inflammation in obesity and diabetes: islet dysfunction and therapeutic opportunity. *Cell Metab* 2013; **17**: 860-872 [PMID: 23747245 DOI: 10.1016/j.cmet.2013.05.001]

114 **DiK B**, Bahcivan E, Eser Faki H, Uney K. Combined treatment with interleukin-1 and tumor necrosis factor-alpha antagonists improve type 2 diabetes in rats. *Can J Physiol Pharmacol* 2018; **96**: 751-756 [PMID: 29558631 DOI: 10.1139/cjpp-2017-0769]

115 **Larsen CM**, Faulenbach M, Vaag A, Ehses JA, Donath MY, Mandrup-Poulsen T. Sustained effects of interleukin-1 receptor antagonist treatment in type 2 diabetes. *Diabetes Care* 2009; **32**: 1663-1668 [PMID: 19542207 DOI: 10.2337/dc09-0533]

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Retrospective Study

Proportion of thyroid cancer and other cancers in the democratic republic of Congo

John Bukasa-Kakamba, Ayrton I Bangolo, Pascal Bayauli, Branly Mbunga, Francis Iyese, Aliocha Nkodila, Ali Atoot, Gaurav Anand, Stacy H Lee, Maimona Chaudhary, Pamela Q Fernandes, Hari PSS Mannam, Adithya Polavarapu, Merajunissa Merajunnissa, Abdullah Azhar, Mohan N Alichetty, Gauravdeep Singh, Georgemar V Arana Jr, Imranjot Sekhon, Manbir Singh, José D Rodriguez-Castro, Adam Atoot, Simcha Weissman, Jean Rene M'buyamba

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John Bukasa-Kakamba, Pascal Bayauli, Francis Iyese, Jean Rene M'buyamba, Endocrine and Metabolic Disorders Department, University of Kinshasa, Kinshasa Kinshasa, Congo

Ayrton I Bangolo, Ali Atoot, Gaurav Anand, Stacy H Lee, Maimona Chaudhary, Pamela Q Fernandes, Hari PSS Mannam, Adithya Polavarapu, Merajunissa Merajunnissa, Abdullah Azhar, Mohan N Alichetty, Gauravdeep Singh, Georgemar V Arana Jr, Imranjot Sekhon, Manbir Singh, José D Rodriguez-Castro, Adam Atoot, Simcha Weissman, Department of Internal Medicine, Palisades Medical Center, North Bergen, NJ 07047, United States

Branly Mbunga, School of Public Health, University of Kinshasa, Kinshasa Kinshasa, Congo

Aliocha Nkodila, Family Medicine, Protestant University of Congo, Kinshasa Kinshasa, Congo

Adam Atoot, Department of Medicine, Hackensack University-Palisades Medical Center, North Bergen, NJ 07047, United States

Corresponding author: Ayrton I Bangolo, MBBS, MD, Doctor, Department of Internal Medicine, Palisades Medical Center, 7600 River Road, North Bergen, NJ 07047, United States. ayrtonbangolo@yahoo.com

Abstract

BACKGROUND

Cancer diagnosis is increasing around the world and in the Democratic Republic of the Congo (DRC). The proportion of thyroid cancer has increased over the past three decades. There are very few studies on cancer epidemiology, and in particular on thyroid cancer in the DRC.

AIM

To establish the most recent proportion of thyroid cancer in the DRC compared to other cancers.

METHODS

This is a retrospective and descriptive study of 6106 consecutive cancer cases

listed in the pathological registers of 4 Laboratories in the city of Kinshasa. This study included all cancer cases recorded in the registers between 2005 and 2019.

RESULTS

From a sample of 6106 patients, including all cancer types, 68.3% cases were female and 31.7% were male. Breast and cervical cancer were the most common types of cancer in women and, prostate and skin cancer were the most common types in men. Thyroid cancer was sixth in proportion in women and eleventh in men compared to all cancers. Papillary carcinoma was the most common of thyroid cancers. Rare cancers such as anaplastic and medullary thyroid carcinomas had a proportion of 7% and 2%, respectively.

CONCLUSION

Newer diagnostic tools led to a surge in cancer diagnoses in the DRC. Thyroid cancer has more than doubled its proportion over the last several decades in the country.

Key Words: Thyroid cancer; Papillary carcinoma; Cancer; Democratic Republic of the Congo; Africa; Proportion

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Core Tip: Cancer diagnosis has been increasing worldwide. This is also true in Africa, particularly in the second biggest African country. However, there are currently no data on cancer in the Democratic Republic of Country (DRC). This study offers the most updated cancer data in general and thyroid cancer in particular in the DRC. Using this current database, more research can be carried out in the country.

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INTRODUCTION

Thyroid pathology is the most common endocrinopathy worldwide[1] and is mostly represented by goiters and nodules[2,3]. Five to ten percent of thyroid nodules are malignant[3-6]. Thyroid cancer only represents 1% of all cancers worldwide[7,8] but has occupied the fifth position among all cancers in France and Canada in women in terms of incidence and twentieth in terms of mortality in 2005[9-11]. The improvement of diagnostic techniques by means of thyroid ultrasound, fine needle aspiration, Computed Tomography scan and detailed histopathological analyses partly explains the increase in incidence of thyroid cancer[12,13]. Despite this increase in incidence, the mortality curve has remained stable over time[10,14].

The Democratic Republic of the Congo (DRC) is a low-income country where there are only 7 pathology laboratories for more than 80 million citizens. Five of these laboratories are located in the capital city of Kinshasa. The typical Congolese meals have been characterized with a low iodine content for decades. Iodine deficiency is a well-known risk factor for thyroid cancer[14,15]. We thus hypothesize that thyroid cancer may be frequent in the DRC but reliable data on cancers in general and thyroid cancer in particular is scarce. The first study addressing thyroid cancer proportion in the DRC was conducted by Mashinda *et al*[16] and it revealed, in women, a thyroid cancer proportion of 0.5% out of all cancers found in the anatomopathological records between 1969 and 2008. Although epidemiologic trend changes are expected to be gradual, the available data now seems dated. The objective of this study is thus to provide more recent thyroid cancer proportion data using the largest series analyzed so far in the DRC.

MATERIALS AND METHODS

This is a retrospective and descriptive study of thyroid cancers and of all types of cancer retrieved from the records of 4 anatomopathological laboratories including that of Kinshasa University Clinics,

National Institute of Biomedical Research, Kinshasa General Hospital (HGRK) and LEBOMA laboratory. All these laboratories are located in the capital city of Kinshasa, a city of nearly 12 million inhabitants. This study included cancers diagnosed in those centers between 2005 and 2019, except for the data obtained from LEBOMA laboratory, which covered from 2015 to 2019. The choice of these centers was governed by the fact that they are the only pathology laboratories in the town of Kinshasa with available data over the period of the study. We calculated the relative proportion of thyroid cancer by dividing the number of thyroid cancers by the number of all types of cancer. It's important to report that calcitonin was not measured preoperatively in patients with thyroid cancer.

The study took into account the following socio-demographic characteristics: Age, gender, year of diagnosis and histopathological diagnosis.

The following types of cancer were taken into account: Breast cancer, cervical cancer, prostate cancer, skin cancer, hematologic cancers, uterine cancer, colon cancer, lung cancer, stomach cancer, bone cancer, thyroid cancer, anorectal cancer, Kaposi sarcoma, soft tissue cancers, eye cancers, ovarian cancer, mouth cancer, vaginal cancer, urinary bladder cancer, laryngeal cancer, nose cancer, peritoneal cancer, liver cancer, renal cancer, vulva cancer, ureteral cancer, nasopharyngeal cancer, intestinal cancer, pancreatic cancer, greater omentum cancer, esophageal cancer, penile cancer, testicular cancer, tonsillar cancer, brain cancer, coecum cancer, vocal cords cancer, ear cancer, parotid glands cancer, duodenal cancer, cancer of the palate, forehead cancer, glottis cancer, trachea cancer, sweat glands cancer, maxillary cancer and splenic cancer.

Data was entered into Excel and transported to Statistical Package for the Social Sciences version 21. Quantitative variables were expressed as mean (+/- SD) or median (+/- interquartile range) for variables that did not have a normal distribution. Qualitative variables were expressed as proportions. Student's *t*-test was used for comparing averages between men and women. χ^2 test or Fisher's exact test was used to compare the difference in proportions between the 2 groups regarding qualitative variables. A P value < 0.05 was of statistical significance.

RESULTS

6106 cancer cases were included in this study. A female predominance was observed with 68.3% of cases *vs* 32.7% for men with a female/male ratio of 4. We noted 106 cases of thyroid cancer, representing 1.7% of the total number of cancer cases. The proportion of all types of cancer according to their location and gender, are grouped together in Table 1.

The most common types of cancer, in decreasing order of proportion were breast cancer, cervical cancer, prostate cancer, skin cancer and lymphoid cancers. Thyroid cancer ranked eleventh in proportion for all cancer types.

The five most common types of cancer in women were breast cancer, cervical cancer, uterine cancer, skin cancer and lymphoid organ (LO) cancer. Thyroid cancer was ranked Sixth with a proportion of 2%.

The five most common types of cancer in men were prostate cancer, skin cancer, LO cancer, lung cancer and colon cancer. For men, thyroid cancer was ranked eleventh (1.1% of all types of cancer).

The male gender was more represented in the age groups ≤ 30 and > 60, while the female gender was more represented in the age groups between 30 and 60 years old. Most cases of cancer in women occur between the ages of 40 and 60 and in men over 50. The number of patients diagnosed with cancer increases with age in both genders.

In the age group up to 40 years old and between 41 and 60 years old, breast cancer was the most common type of cancer. In the age group of over 61, prostate cancer was the most common. Thyroid cancer occupied the sixth position in the age group under 41 years, the tenth in the age group between 41 years and 60 years and the sixteenth in the age group over 60 years old. Figure 1 represents cancer cases' distribution based on age and gender.

The frequencies of cancer cases according to age groups are found in Table 2.

Papillary carcinoma was the most common type of thyroid cancer, representing 67% of all thyroid cancer cases followed by the follicular type in 21% of cases. The anaplastic type occupied the third, lymphoma the fourth and medullary cancer the fifth position.

The frequencies of the different types of thyroid cancer cases in this series are found in Table 3.

DISCUSSION

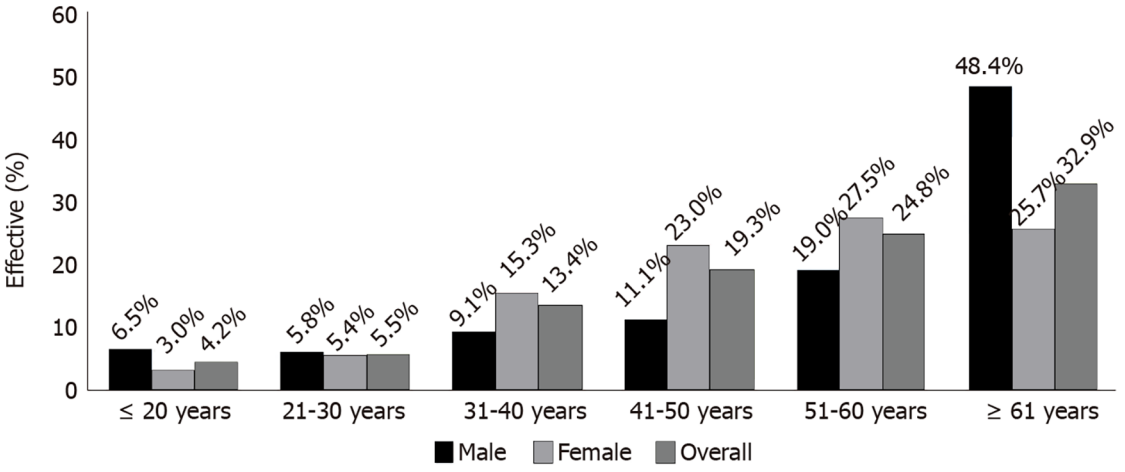
The main objective of this research was to establish the proportion of thyroid cancer in the Congolese population, secondarily aiming to identify the most frequent types of cancer. Our series included 6106 cases, of which 68.3 % were female and 31.7% were male. This predominance of the female gender was also found in the series of Mashinda who studied the proportion of cancer in the urban setting of Kinshasa[16] and it was also the case for the Lukanu series, which included cases from a rural setting of Congo in Kimpese[17]. These are the first two epidemiological studies analyzing neoplastic diseases carried out in the DRC and which preceded our research.

Table 1 Cancer distribution by affected organs and gender

	All, *n* = 6106	Females, *n* = 4169 (68.3%)	Males, *n* = 1937 (31.7%)
Breast	1631 (26.7)	1560 (37.4)	71 (3.7)
Cervix	1138 (18.6)	1138 (27.3)	-
Prostate	678 (11.1)	-	678 (35)
Skin	356 (5.8)	186 (4.5)	170 (8.8)
Blood and LO	206 (3.4)	95 (2.3)	111 (5.7)
Uterus	196 (3.2)	196 (4.7)	-
Colon	173 (2.8)	92 (2.2)	81 (4.2)
Lungs	161 (2.6)	60 (1.4)	101 (5.2)
Stomach	112 (1.8)	45 (1.1)	67 (3.5)
Bone	110 (1.8)	58 (1.4)	52 (2.7)
Thyroid	106 (1.7)	84 (2)	22 (1.1)
Anus/rectum	97 (1.6)	51 (1.2)	46 (2.4)
KS	87 (1.4)	22 (0.5)	65 (3.4)
Soft tissue	87 (1.4)	52 (1.2)	35 (1.8)
Eye	77 (1.3)	35 (0.8)	42 (2.2)
Ovary	77 (1.3)	77 (1.8)	-
Mouth	76 (1.2)	44 (1.1)	32 (1.7)
Vagina	75 (1.2)	75 (1.8)	-
Urinary bladder	73 (1.2)	32 (0.8)	41 (2.1)
Larynx	65 (1.1)	11 (0.3)	54 (2.8)
Nose	62 (1.0)	28 (0.7)	34 (1.8)
Peritoneum	51 (0.8)	24 (0.6)	27 (1.4)
Liver	49 (0.8)	24 (0.6)	25 (1.3)
Kidneys	41 (0.7)	21 (0.5)	20 (1)
Vulva	41 (0.7)	41 (1)	-
Ureter	33 (0.5)	14 (0.3)	19 (1)
Pharynx/nasopharynx	33 (0.5)	19 (0.4)	14 (0.7)
Intestines	29 (0.5)	16 (0.4)	13 (0.7)
Pancreas	29 (0.5)	13 (0.3)	16 (0.8)
Greater omentum	22 (0.4)	15 (0.4)	7 (0.4)
Esophagus	21 (0.3)	8 (0.2)	13 (0.7)
Penis	20 (0.3)	-	20 (1)
Testicles	20 (0.3)	-	20 (1)
Tonsils	13 (0.2)	5 (0.1)	8 (0.4)
Brain	10 (0.2)	6 (0.1)	4 (0.2)
Coecum	10 (0.2)	4 (0.1)	6 (0.3)
Vocal cords	8 (0.1)	1 (0.0)	7 (0.4)
Ears	7 (0.1)	5 (0.1)	2 (0.1)
Parotid glands	7 (0.1)	2 (0.0)	5 (0.3)
Duodenum	6 (0.1)	2 (0.0)	4 (0.2)
Palate	4 (0.1)	4 (0.1)	0 (0.0)

Forehead	2 (0.0)	1 (0.0)	1 (0.1)
Glottis	2 (0.0)	0 (0.0)	2 (0.1)
Trachea	2 (0.0)	1 (0.0)	1 (0.1)
Sweat glands	1 (0.0)	1 (0.0)	0 (0.0)
Maxillary	1 (0.0)	1 (0.0)	0 (0.0)
Spleen	1 (0.0)	0 (0.0)	1 (0.1)

LO: Lymphoid organ; KS: Kaposi Sarcoma.



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Figure 1 Cancer distribution based on age and gender.

Female predominance in overall cancer diagnosis was observed in the DRC, a similar trend was also observed in Brazil[18]. The cascade of sexual hormone activity, in particular estrogens and the aromatization of androgens *via* Mitogen-activated protein kinase, causing the decline of immune cells and promoting the proliferation of cancer cells and the inhibition of apoptotic activity can explain the female predominance[19,20]. However, some European series have found a male predominance[21-25].

The male gender was more represented in the age groups up to 30 years and over 60 years; on the other hand, the female gender was more represented in the age groups between 30 and 60 years. Most cancers in women occurred between the ages of 40 and 60 and in men over 50. Overall, 77% of cancer patients were over 40 years of age. Most series around the world, according to which the older the age the greater the probability of developing a neoplastic disease[26,27].

In our series, breast cancer occupies the first position in terms of proportion of cancer in women and cervical cancer occupies the second position. However, in the series published by Mashinda *et al*[16], cervical cancer was the most frequent followed by breast cancer. This difference can be explained by the methodology, the study period, the progress of the national policy on cancer screening in women and by the development of diagnostic means. Mashinda studied the records of two pathology laboratories, while we researched the records of 4 pathology laboratories. Mashinda analyzed results from 1965 to 2008, while we studied data between 2005 and 2019. We must consider that the means of raising awareness have evolved and the educational level of the population has increased over time. All these parameters can explain this difference. The findings of our study are similar to the Lukanu series; breast cancer was the most frequent followed by cervical cancer[17]. Our results also mirrored those found in several African and worldwide series[28-31].

When we consider both sexes, breast cancer was the most frequent type of cancer in our series, this result being similar to those in the literature[29].

Regarding cancer in male patients, prostate cancer was the most frequent in our series. This result was similar to the series of Lukanu[17]. On the other hand, Mashinda found lymphoid organ cancer as the most frequent followed by prostate cancer[16]. Our results are similar to those found in the literature [30]. There has been an improvement in the awareness of the Congolese population regarding prostate cancer over the past two decades.

Thyroid cancer is the most common type of cancer of the endocrine system[32]. Our series found a proportion of 1.7%. In women, the proportion is 2% and it is 1.1% in men, with a female/male ratio of 4. The series described by Mashinda *et al*[16] found a thyroid cancer proportion of 0.5% in women. The

Table 2 Cancer distribution by age group

Cancers	≤ 40 yr, *n* (1409)	%	41-60 yr, *n* (2689)	%	≥ 61 yr, *n* (2008)	%
Prostate	25	1.8	122	4.5	531	26.4
Cervical	156	11.1	611	22.7	371	18.5
Breast	403	28.6	895	33.3	333	16.6
Skin	141	10.0	133	4.9	82	4
Uterus	22	1.6	107	4.0	67	3.3
Lungs	30	2.1	64	2.4	67	3.3
Blood and LO	106	7.5	48	1.8	52	2.6
Colon	37	2.6	92	3.4	44	2.2
Stomach	19	1.3	60	2.2	33	1.6
KS	32	2.3	24	0.9	31	1.5
Soft tissue	27	1.9	30	1.1	30	1.5
Vessels	13	0.9	32	1.2	28	1.4
Bones	51	3.6	33	1.2	26	1.3
Vagina	16	1.1	33	1.2	26	1.3
Larynx	18	1.3	21	0.8	26	1.3
Anus-rectum	27	1.9	45	1.7	25	1.2
Thyroid	37	2.6	45	1.7	24	1.2
Mouth	28	2.0	24	0.9	24	1.2
Liver	12	0.9	19	0.7	18	0.9
Ureter	3	0.2	12	0.4	18	0.9
Vulva	4	0.3	20	0.7	17	0.8
Ovary	25	1.8	36	1.3	16	0.8
Peritoneum	12	0.9	23	0.9	16	0.8
Nose	26	1.8	23	0.9	13	0.6
Eye	35	2.5	30	1.1	12	0.6
Esophagus	5	0.4	6	0.2	10	0.5
Testicles	8	0.6	2	0.1	10	0.5
Pharynx	14	1.0	8	0.3	9	0.4
Pancreas	5	0.4	16	0.6	8	0.4
Intestines	7	0.5	15	0.6	7	0.3
Kidney	23	1.6	11	0.4	7	0.3
Vocal cords	1	0.1	2	0.1	5	0.2
Greater omentum	7	0.5	11	0.4	4	0.2
Penis	5	0.4	12	0.4	3	0.1
Duodenum	1	0.1	2	0.1	3	0.1
Tonsils	4	0.3	7	0.3	2	0.1
Coecum	5	0.4	3	0.1	2	0.1
Ears	4	0.3	1	0.0	2	0.1
Palate	1	0.1	1	0.0	2	0.1
Parotid glands	3	0.2	3	0.1	1	0.0
Trachea	1	0.1	0	0.0	1	0.0

Maxillary	0	0.0	0	0.0	1	0.0
Spleen	0	0.0	0	0.0	1	0.0
Brain	6	0.4	4	0.1	0	0.0
Glottis	0	0.0	2	0.1	0	0.0
Nasopharynx	1	0.1	1	0.0	0	0.0
Forehead	2	0.1	0	0.0	0	0.0
Sweat glands	1	0.1	0	0.0	0	0.0

LO: Lymphoid organ; KS: Kaposi Sarcoma.

Table 3 Frequency and distribution of the cases of thyroid cancer according to histological type

Variables	Number of cases	Percentage (%)
Histology		
Papillary carcinoma	71	67.0
Follicular carcinoma	23	21.7
Anaplastic carcinoma	8	7.5
Lymphoma	3	2.8
Medullary carcinoma	1	0.9

proportion of thyroid cancer in women in our series compared to that of Mashinda's is multiplied by 4, a female predominance that is confirmed in the literature[33,34]. Thyroid cancer occupied the sixth position of all listed cancers among women in our series, whereas it is the 5th most frequent cancer in women worldwide[35].

The increase in thyroid cancer proportion has also been observed in several studies around the world over the past three decades[10]. The mechanisms underlying this increase have not yet been elucidated. However, nutritional, hormonal, anthropometric, environmental, and other factors are suspected. Many authors also consider that excessive iodine intake, and the development and accessibility of diagnostic tools participate to the increase in diagnosis[36,37].

Our study found that nearly 7% of thyroid cancers were anaplastic and 1% were medullary, while these cancers are rare in the literature[38]. This can be considered as a particularity of the DRC regarding thyroid cancer, especially since calcitonin is not generally measured in the assessment of thyroid nodules or preceding thyroidectomy.

It is known that 90% of thyroid cancers are differentiated and have good prognosis and that only 5% to 10% are undifferentiated and have therefore a bad prognosis[39,40]. This high proportion of undifferentiated cancers in our series constitutes a particularity of the Congo. This can be explained by the fact that, undifferentiated thyroid cancers, given their aggressive behavior, are more likely to warrant a surgical evaluation. Thyroid cancers in our study originated from surgical pathology reports. Nevertheless, this particularity requires more in-depth studies to better understand the causes and mechanisms.

Since iodine deficiency in the soil is considered a risk factor for anaplastic cancer[41], the question to be raised is whether iodine deficiency could be responsible for this higher proportion of undifferentiated cancers. knowing that iodine saturation in the Congo was only obtained in 1993[42]. Another potential mechanism is that initially differentiated cancers have lost differentiation over time[43] due to late diagnosis.

This work has the limitations of retrospective studies. In addition, it is biased due to the fact that we only took into consideration the patients who had carried out the anatomopathology while those who had not carried out one, were not included in this study, this may have influenced a high proportion of cancers and certain histological types. Finally, limitations in diagnostic facilities in data reporting in a resource-poor healthcare facility are also potentially limiting.

Despite these limitations, this work gives a scoping vision of cancer in the DRC and in particular of thyroid cancer. It has established the frequencies of different forms of cancer in a country where cancer data are rare.

CONCLUSION

Cancer diagnosis is on the rise in the DRC and the proportion of thyroid cancer as compared to total number of cancers has doubled over the period from 2005 to 2019. A marked female predominance was observed. Papillary thyroid cancer is the most frequent type of thyroid cancer followed by follicular carcinoma. There is a high proportion of undifferentiated thyroid cancers such as anaplastic carcinomas, long recognized as rare carcinomas. Breast cancer is the most common of all types of cancer, followed by cervical cancer. Prostate cancer is the most common type of cancer in men. Thyroid cancer ranked sixth most common cancer in women and eleventh most common in men. This study establishes the most recent and updated proportion of thyroid cancer in the second-largest African country.

ARTICLE HIGHLIGHTS

Research background

Cancer diagnosis has been increasing worldwide and in Africa as well, particularly in the Democratic Republic of Congo (DRC). However, there are currently no studies addressing the proportion of different cancers in the DRC, and in particular thyroid cancer.

Research motivation

The main motivation of this study was to identify the proportions of different cancers in the DRC and in particular thyroid cancer.

Research objectives

The purpose of this study was to analyze different epidemiologic characteristics of thyroid cancers in the second-largest African country while establishing the proportions of all cancers in the country.

Research methods

This is a retrospective and descriptive study of 6106 consecutive cancer cases listed in the pathological registers of 4 Laboratories in the city of Kinshasa. This study included all cancer cases recorded in the registers between 2005 and 2019.

Research results

In our series two third of cancer patients were females. Breast is the most common cancer in females while prostate cancer is the most common among their male counterparts. Thyroid cancer was ranked sixth in occurrence and a higher proportion of anaplastic thyroid cancer was encountered.

Research conclusions

Female patients seem to be more affected by cancer than their male counterparts. Rare anaplastic thyroid cancers which are often associated with a dismal prognosis, although rare in the literature, are found in higher proportion in the DRC. A surge of all cancers was also observed owing to the advances in diagnostic tools used.

Research perspectives

Oncology and cancer research in the DRC remains an unexplored area. There is a serious paucity of data of any cancer in the country. This study offers the most updated data on cancer in the second-largest African country. This study paves the way for future prospective studies in the country, helping to identify groups at higher risk and shaping the national guidelines.

FOOTNOTES

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Country/Territory of origin: United States

ORCID number: Ayrton I Bangolo 0000-0002-2133-2480; Adam Atoot 0000-0001-7037-3687; Simcha Weissman 0000-0002-0796-6217.

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REFERENCES

1 **Unnikrishnan AG**, Menon UV. Thyroid disorders in India: An epidemiological perspective. *Indian J Endocrinol Metab* 2011; **15**: S78-S81 [PMID: 21966658 DOI: 10.4103/2230-8210.83329]
2 **Hegedüs L**. Clinical practice. The thyroid nodule. *N Engl J Med* 2004; **351**: 1764-1771 [PMID: 15496625 DOI: 10.1056/nejmcp031436]
3 **Bouaity B**, Darouassi Y, Chihani M, Touati MM, Ammar H. [Analysis of predictors of malignancy of nodular goiters: about 500 cases]. *Pan Afr Med J* 2016; **23**: 88 [PMID: 27222685 DOI: 10.11604/pamj.2016.23.88.8405]
4 **Wémeau JL**, Sadoul JL, d'Herbomez M, Monpeyssen H, Tramalloni J, Leteurtre E, Borson-Chazot F, Caron P, Carnaille B, Léger J, Do C, Klein M, Raingeard I, Desailloud R, Leenhardt L. Guidelines of the French society of endocrinology for the management of thyroid nodules. *Ann Endocrinol (Paris)* 2011; **72**: 251-281 [PMID: 21782154 DOI: 10.1016/j.ando.2011.05.003]
5 **Belfiore A**, La Rosa GL, Padova G, Sava L, Ippolito O, Vigneri R. The frequency of cold thyroid nodules and thyroid malignancies in patients from an iodine-deficient area. *Cancer* 1987; **60**: 3096-3102 [PMID: 3677033 DOI: 10.1002/1097-0142(19871215)60:12<3096::aid-cncr2820601240>3.0.co;2-v]
6 **Rago T**, Chiovato L, Aghini-Lombardi F, Grasso L, Pinchera A, Vitti P. Non-palpable thyroid nodules in a borderline iodine-sufficient area: detection by ultrasonography and follow-up. *J Endocrinol Invest* 2001; **24**: 770-776 [PMID: 11765046 DOI: 10.1007/bf03343926]
7 **Haugen BR**, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid* 2016; **26**: 1-133 [PMID: 26462967 DOI: 10.1089/thy.2015.0020]
8 **Touati MM**, Aljalil A, Darouassi Y, Chihani M, Lahkim M, Fihri JA, Bouaity B, Ammar H. [Thyroid carcinoma: epidemiological, clinical and therapeutic profiles, about 102 cases]. *Pan Afr Med J* 2015; **21**: 59 [PMID: 26405495 DOI: 10.11604/pamj.2015.21.59.5688]
9 **Colonna M**, Bossard N, Guizard AV, Remontet L, Grosclaude P; le réseau FRANCIM. Descriptive epidemiology of thyroid cancer in France: incidence, mortality and survival. *Ann Endocrinol (Paris)* 2010; **71**: 95-101 [PMID: 20036351 DOI: 10.1016/j.ando.2009.11.006]
10 **Kilfoy BA**, Zheng T, Holford TR, Han X, Ward MH, Sjodin A, Zhang Y, Bai Y, Zhu C, Guo GL, Rothman N. International patterns and trends in thyroid cancer incidence, 1973-2002. *Cancer Causes Control* 2009; **20**: 525-531 [PMID: 19016336 DOI: 10.1007/s10552-008-9260-4]
11 **Government of Canada SC**. Changing trends in thyroid cancer incidence in Canada: A histologic examination, 1992 to 2016 [Internet]. 2020. Available from: https://www150.statcan.gc.ca/n1/pub/82-003-x/2020001/article/00002-eng.htm
12 **Jung CK**, Little MP, Lubin JH, Brenner AV, Wells SA Jr, Sigurdson AJ, Nikiforov YE. The increase in thyroid cancer incidence during the last four decades is accompanied by a high frequency of BRAF mutations and a sharp increase in RAS mutations. *J Clin Endocrinol Metab* 2014; **99**: E276-E285 [PMID: 24248188 DOI: 10.1210/jc.2013-2503]
13 **Seib CD**, Sosa JA. Evolving Understanding of the Epidemiology of Thyroid Cancer. *Endocrinol Metab Clin North Am* 2019; **48**: 23-35 [PMID: 30717905 DOI: 10.1016/j.ecl.2018.10.002]
14 **Leenhardt L**, Bernier MO, Boin-Pineau MH, Conte Devolx B, Maréchaud R, Niccoli-Sire P, Nocaudie M, Orgiazzi J, Schlumberger M, Wémeau JL, Chérie-Challine L, De Vathaire F. Advances in diagnostic practices affect thyroid cancer incidence in France. *Eur J Endocrinol* 2004; **150**: 133-139 [PMID: 14763910 DOI: 10.1530/eje.0.1500133]
15 **Cardis E**, Kesminiene A, Ivanov V, Malakhova I, Shibata Y, Khrouch V, Drozdovitch V, Maceika E, Zvonova I, Vlassov O, Bouville A, Goulko G, Hoshi M, Abrosimov A, Anoshko J, Astakhova L, Chekin S, Demidchik E, Galanti R, Ito M, Korobova E, Lushnikov E, Maksioutov M, Masyakin V, Nerovnia A, Parshin V, Parshkov E, Piliptsevich N, Pinchera A, Polyakov S, Shabeka N, Suonio E, Tenet V, Tsyb A, Yamashita S, Williams D. Risk of thyroid cancer after exposure to

131I in childhood. *J Natl Cancer Inst* 2005; **97**: 724-732 [PMID: 15900042 DOI: 10.1093/jnci/dji129]
16 **Mashinda KD**, Kayembe KP, Mapatano MA. Prévalence du cancer en République Démocratique du Congo: données anatomopathologiques recueillies aux Cliniques Universitaires et à l'Hôpital Général de Référence de Kinshasa Cancer prevalence in Democratic Republic of the Congo: anatomopathologic. *Annales africaines de médecine* 2012; **5**: 3
17 **Lukanu NP**, Ntontolo NP, Diakengua V, Kalombo C, Nyambu J, Landu J, Nlandu J, Atungu P, Nsiangana Z, Nkodila A. Epidemiology of Cancer in Rural Congo: Case of IME Kimpese Hospital, Democratic Republic of Congo. *J Cancer Ther* 2021; **12**: 127 [DOI: 10.4236/jct.2021.123014]
18 **Kolankiewicz ACB**, de Souza Magnago TSB, Dos Santos Dullius AI, De Domenico EBL. Liens entre les variables dé mographiques, économiques et cliniques et les symptômes rapportés par les patients en cours de traitement contre le cancer. *Can Oncol Nurs J* 2017; **27**: 375-385 [PMID: 31148733 DOI: 10.5737/23688076274375385]
19 **Irelli A**, Sirufo MM, D'Ugo C, Ginaldi L, De Martinis M. Sex and Gender Influences on Cancer Immunotherapy Response. *Biomedicines* 2020; **8** [PMID: 32708265 DOI: 10.3390/biomedicines8070232]
20 **Özdemir BC**, Dotto GP. Sex Hormones and Anticancer Immunity. *Clin Cancer Res* 2019; **25**: 4603-4610 [PMID: 30890551 DOI: 10.1158/1078-0432.CCR-19-0137]
21 **Dorak MT**, Karpuzoglu E. Gender differences in cancer susceptibility: an inadequately addressed issue. *Front Genet* 2012; **3**: 268 [PMID: 23226157 DOI: 10.3389/fgene.2012.00268]
22 **Dyba T**, Randi G, Bray F, Martos C, Giusti F, Nicholson N, Gavin A, Flego M, Neamtiu L, Dimitrova N, Negrão Carvalho R, Ferlay J, Bettio M. The European cancer burden in 2020: Incidence and mortality estimates for 40 countries and 25 major cancers. *Eur J Cancer* 2021; **157**: 308-347 [PMID: 34560371 DOI: 10.1016/j.ejca.2021.07.039]
23 **Ketfi A**, Zanoun N, Laouedj I, Gharnaout M, Fraga S. [Primary lung cancer and occupational exposure in a North African population]. *Pan Afr Med J* 2020; **37**: 120 [PMID: 33425153 DOI: 10.11604/pamj.2020.37.120.21755]
24 **Cao B**, Hill C, Bonaldi C, León ME, Menvielle G, Arwidson P, Bray F, Soerjomataram I. Cancers attributable to tobacco smoking in France in 2015. *Eur J Public Health* 2018; **28**: 707-712 [PMID: 29741657 DOI: 10.1093/eurpub/cky077]
25 **Nga WTB**, Eloumou SAFB, Engbang JPN, Bell EMD, Mayeh AMM, Atenguena E, Biwole ME, Ayissi GBN, Kenfack G, Noah DN, Luma HN, Sone AM, Ndom P, Ndam ECN. [Prognosis and survival of esophageal cancer in Cameroon: a prognostic study]. *Pan Afr Med J* 2019; **33**: 73 [PMID: 31448035 DOI: 10.11604/pamj.2019.33.73.16112]
26 **Ndahindwa V**, Ngendahayo L, Vyankandondera J. Aspects Epidemiologiques Et Anatomopathologiques Des Cancers Dans Les Centres Hospitaliers Universitaires (Chu) Du Rwanda. *RMJ* 2012; **69**: 10
27 **Chase D**. Letter in response to the original article: "Evaluation of femoral approach to coronary sinus catheterisation in electrophysiological and ablation procedures: Single centre experience" authored by Osama Abdel Atty, Mohamed Morsy and Mark M. Gallagher (Journal of the Saudi Heart Association, Volume 23, Issue 4, October 2011, pp. 213-216). *J Saudi Heart Assoc* 2012; **24**: 145 [PMID: 23960685 DOI: 10.1016/j.jsha.2012.01.001]
28 **Airtum Working Group**. [Numbers (Airtum Working Group). Survival prospects change with time]. *Epidemiol Prev* 2008; **32**: 136 [PMID: 18828550]
29 **Fwelo P**, Nwosu KOS, Adekunle TE, Afolayan O, Ahaiwe O, Ojaruega AA, Nagesh VK, Bangolo A. Racial/ethnic and socioeconomic differences in breast cancer surgery performed and delayed treatment: mediating impact on mortality. *Breast Cancer Res Treat* 2023; **199**: 511-531 [PMID: 37052762 DOI: 10.1007/s10549-023-06941-z]
30 **Organisation mondiale de la Sante**. Breast Cancer. 2021. Available from: https://www.who.int/fr/news-room/fact-sheets/detail/breast-cancer
31 **Chbani L**, Hafid I, Berraho M, Mesbahi O, Nejjari C, Amarti A. [Epidemiological and pathological features of cancer in Fez Boulemane region, Morocco]. *East Mediterr Health J* 2013; **19**: 263-270 [PMID: 23879078]
32 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. *CA Cancer J Clin* 2008; **58**: 71-96 [PMID: 18287387 DOI: 10.3322/CA.2007.0010]
33 **Kilfoy BA**, Devesa SS, Ward MH, Zhang Y, Rosenberg PS, Holford TR, Anderson WF. Gender is an age-specific effect modifier for papillary cancers of the thyroid gland. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 1092-1100 [PMID: 19293311 DOI: 10.1158/1055-9965.EPI-08-0976]
34 **Moses W**, Weng J, Kebebew E. Prevalence, clinicopathologic features, and somatic genetic mutation profile in familial versus sporadic nonmedullary thyroid cancer. *Thyroid* 2011; **21**: 367-371 [PMID: 21190444 DOI: 10.1089/thy.2010.0256]
35 **Worldwide cancer data**. World Cancer Research Fund International [Internet]. WCRF International. [cite 16 in 2021]. Available from: https://www.wcrf.org/dietandcancer/worldwide-cancer-data/
36 **Jegerlehner S**, Bulliard JL, Aujesky D, Rodondi N, Germann S, Konzelmann I, Chiolero A; NICER Working Group. Overdiagnosis and overtreatment of thyroid cancer: A population-based temporal trend study. *PLoS One* 2017; **12**: e0179387 [PMID: 28614405 DOI: 10.1371/journal.pone.0179387]
37 **Dal Maso L**, Bosetti C, La Vecchia C, Franceschi S. Risk factors for thyroid cancer: an epidemiological review focused on nutritional factors. *Cancer Causes Control* 2009; **20**: 75-86 [PMID: 18766448 DOI: 10.1007/s10552-008-9219-5]
38 **Wemeau JL**, Do Cao C. [Anaplastic thyroid carcinoma]. *Ann Endocrinol (Paris)* 2008; **69**: 174-180 [PMID: 18423422 DOI: 10.1016/j.ando.2008.02.003]
39 **Rego-Iraeta A**, Pérez-Méndez LF, Mantinan B, Garcia-Mayor RV. Time trends for thyroid cancer in northwestern Spain: true rise in the incidence of micro and larger forms of papillary thyroid carcinoma. *Thyroid* 2009; **19**: 333-340 [PMID: 19355823 DOI: 10.1089/thy.2008.0210]
40 **Zhu J**, Sun K, Wang J, He Y, Li D, Liu S, Huang Y, Zhang M, Song B, Liao X, Liang H, Zhang Q, Shi M, Guo L, Zhou Y, Lin Y, Lu Y, Tuo J, Xia Y, Sun H, Xiao H, Ji Y, Yan C, Qiao J, Zeng H, Zheng R, Zhang S, Chang S, Wei W. Clinicopathological and surgical comparisons of differentiated thyroid cancer between China and the USA: A multicentered hospital-based study. *Front Public Health* 2022; **10**: 974359 [PMID: 36249201 DOI: 10.3389/fpubh.2022.974359]
41 **Kim J**, Gosnell JE, Roman SA. Geographic influences in the global rise of thyroid cancer. *Nat Rev Endocrinol* 2020; **16**: 17-29 [PMID: 31616074 DOI: 10.1038/s41574-019-0263-x]
42 **Banza BI**, Lumbu JB, Donnen P, Twite EK, Kwete DM, Kazadi CM, Ozoza JO, Habimana L, Kalenga PM, Robert A.

[Iodine concentration in cooking salt consumed in Lubumbashi and the iodine status of vulnerable people: case study of pregnant women living in underprivileged areas]. *Pan Afr Med J* 2016; **23**: 129 [PMID: 27279956 DOI: 10.11604/pamj.2016.23.129.7645]

43 **Harach HR**, Galíndez M, Campero M, Ceballos GA. Undifferentiated (anaplastic) thyroid carcinoma and iodine intake in Salta, Argentina. *Endocr Pathol* 2013; **24**: 125-131 [PMID: 23666798 DOI: 10.1007/s12022-013-9248-9]

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Role of children in the Bulgarian COVID-19 epidemic: A mathematical model study

Latchezar Tomov, Hristiana Batselova, Snezhina Lazova, Borislav Ganev, Iren Tzocheva, Tsvetelina Velikova

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Latchezar Tomov, Department of Informatics, New Bulgarian University, Sofia 1618, Bulgaria

Hristiana Batselova, Department of Epidemiology and Disaster Medicine, Medical University, University Hospital "St George", Plovdiv 6000, Bulgaria

Snezhina Lazova, Borislav Ganev, Department of Pediatric, University Hospital "N. I. Pirogov", Sofia 1606, Bulgaria

Snezhina Lazova, Department of Healthcare, Faculty of Public Health, Medical University of Sofia, Sofia 1527, Bulgaria

Iren Tzocheva, Department of Pediatric, Medical Faculty, University Hospital "N. I. Pirogov", Sofia 1606, Bulgaria

Tsvetelina Velikova, Medical Faculty, Sofia University, St. Kliment Ohridski, Sofia 1407, Bulgaria

Corresponding author: Latchezar Tomov, PhD, Academic Research, Assistant Professor, Department of Informatics, New Bulgarian University, Montevideo 21 Street, Sofia 1618, Bulgaria. luchesart@gmail.com

Abstract

BACKGROUND

The coronavirus disease 2019 (COVID-19) pandemic affects all aspects of our lives, including children. With the advancement of the pandemic, children under five years old are at increased risk of hospitalization relative to other age groups. This makes it paramount that we develop tools to address the two critical aspects of preserving children's health – new treatment protocols and new predictive models. For those purposes, we need to understand better the effects of COVID-19 on children, and we need to be able to predict the number of affected children as a proportion of the number of infected children. This is why our research focuses on clinical and epidemiological pictures of children with heart damage post-COVID, as a part of the general picture of post-COVID among this age group.

AIM

To demonstrate the role of children in the COVID-19 spread in Bulgaria and to test the hypothesis that there are no secondary transmissions in schools and from children to adults.

METHODS

Our modeling and data show with high probability that in Bulgaria, with our current measures, vaccination strategy and contact structure, the pandemic is driven by the children and their contacts in school.

RESULTS

This makes it paramount that we develop tools to address the two critical aspects of preserving children's health – new treatment protocols and new predictive models. For those purposes, we need to understand better the effects of COVID-19 on children, and we need to be able to predict the number of affected children as a proportion of the number of infected children. This is why our research focuses on clinical and epidemiological pictures of children with heart damage post-COVID, as a part of the general picture of post-Covid among this age group.

CONCLUSION

Our modeling rejects that hypothesis, and the epidemiological data supports that. We used epidemiological data to support the validity of our modeling. The first summer wave in 2020 from the listed here school proms endorse the idea of transmissions from students to teachers.

Key Words: COVID-19; Pandemic; Children; Cardiac involvement; Multisystem inflammation in children; ARIMA; Time-series modeling; Regression model

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Core Tip: The lack of vaccination strategy accelerates the spread of coronavirus disease 2019 among children and accelerate the spread among people below 50 years. Based on the latter hypothesis and the other three: (1) Disease spreads from children to adults – either directly to parents and grandparents or *via* network spread to different age groups; (2) the spread among children accelerates with the increasing R0 of different dominating viral variants; and (3) vaccinations among adults accelerate the spread among the less vaccinated group of children, we outlined the reasons for this age-restricted acceleration of the spread after the delta wave.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic impacts all aspects of our lives, including the increased burden on the healthcare system, altered work, education, social life, etc. The pandemic also affects children. They are at increased risk for type 1 diabetes post-COVID, even more, pronounced in children than the adult patients[1]. Additionally, they have persistent lung damage[2]. With the advancement of the pandemic, children under five years old are at increased risk of hospitalization relative to other age groups that have immunity from vaccination and/or infection[3]. This makes it paramount that we develop tools to address the two critical aspects of preserving children's health – new treatment protocols and new predictive abilities. For those purposes, we need to understand better the effects of COVID on children, and we need to be able to predict the number of affected children as a proportion of the number of infected children. This is why our research focuses on clinical and epidemiological pictures of children with heart damage post-COVID, as a part of the general picture of post-COVID among this age group.

COVID-19 and children

Although severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection was previously considered clinically milder in children compared to adults, the severe consequences of COVID-19, known as multisystem inflammation in children (MIS-C), has become increasingly significant with the accumulation of practical experience worldwide. As we recently published, MIS-C is associated mainly with late post-viral immunological responses leading to multiorgan impairment and failure[4,5].

Except for liver involvement, cardiac damage is also typical in children after asymptomatic and symptomatic COVID-19, as myocardial, pericardial, and/or coronary manifestations. Recently, we presented the results from the retrospective analysis of pediatric patients with cardiovascular system

involvement in 31.4% of confirmed COVID-19 cases admitted to the hospital[6,7]. The most common clinical features were acute myocarditis with severe contractile dysfunction, acute heart failure, and left main coronary artery dilation; systolic dysfunction, dilation and regional hypokinesia of the left ventricle; acute vasculitis with transient dilation of the coronary arteries; pericarditis as part of a pronounced polyserositis syndrome[6]. It has been demonstrated that children with pre-existing comorbidities (cardiovascular diseases, cancer, renal failure) are at risk for severe COVID-19[8,9].

Fortunately, the course of the disease after the complex treatment (immunomodulatory therapy with intravenous immunoglobulins and corticosteroids, inotropic drugs and anticoagulants) was favorable in all patients. Furthermore, although the COVID-19 therapy is non-specific, to some extent in some cases it may lead to further complications (*i.e.*, liver injury, *etc.*)[10].

In the initial phase of the Covid-19 pandemic, some studies claimed that children rarely get COVID-19 and do not spread as much as adults *via* schools[11]. The airborne nature of the disease was also disputed[12], commenting also the short-range aerosol transmission and while masking will not decrease exposure to the virus to zero, it offers incremental benefits that, when combined with other strategies, will lower the risk of infection even more.

For this reason, very few preventive measures in schools in countries such as Bulgaria were implemented in 2020[13]. Transmission from children to adults was deemed secondary to the transmission from adults to children. After the first big wave in September to December 2020, masks were mandated in grades 5 and above but not enforced, with arguably low adherence, less than the general adherence[14] due to mask removal in breaks between classes.

We use mathematical modeling (time series analysis) to test the following hypotheses in the case of Bulgaria and to show the price of the lack of policy in schools: (1) The possibility of emerging variants to accelerate their spread among people below 50 years, thus compensating for the effect of acquired partial herd immunity; (2) disease spreads from children to adults – either directly to parents and grandparents or *via* network spread to different age groups; (3) the spread among children accelerates with the increasing R0 of different dominating viral variants; and (4) vaccinations among adults accelerate the spread among the less vaccinated group of children. Our modeling can be used for both explanations of dependency and short-term prediction of a future number of cases. It is also potentially applicable to small datasets, such as the MIS-C cases at Pirogov Hospital, as we show in this paper.

Epidemiological data for spread among age groups in Bulgarian children

With starting the pandemic in Bulgaria, children were mostly speared from infection. On the one hand, it was thought that COVID-19 took a mild course in children. On the other hand, the testing was at the lowest rate among children. Up to now, the total number of infected children (0-18 years) is 103743[15].

We aimed to demonstrate the role of children in the COVID-19 spread in Bulgaria and to test the hypothesis that there are no secondary transmissions in schools and from children to adults. Our modeling rejects that hypothesis, and the epidemiological data supports that. We use epidemiological data to support the validity of our modeling. The first summer wave in 2020 from the listed here school proms endorses the idea of transmissions from students to teachers.

MATERIALS AND METHODS

Statistical modeling - methods and hypotheses

In our previous research[16], we used ARIMA models to predict deaths weekly from new cases among different age groups. The approach is suitable for time series analysis with time-varying effective reproductive number Rt. Moreover, it allows us to regress against other predictors to test some hypotheses which set of factors influences the spread of the disease. For children, we have several hypotheses.

The most important one is the significant influence of school closure on the number of new cases. The second in order of importance is that the disease spreads from children to adults – either directly to parents and grandparents or *via* network spread to different age groups. The third hypothesis is that the spread among children accelerates with the increasing R0 of different dominating viral variants. Since the R0, or basic reproduction number[17], is the number of secondary cases of a disease that result from the emergence of a single index case in a population of uniformly dispersed susceptible people, therefore, if the R0 is less than one, it indicates that the epidemic is under control; if it is larger than one, it indicates that the disease is spreading.

The fourth hypothesis is that vaccinations among adults accelerate the spread among the less vaccinated group of children. By acceleration, we mean the increased proportion of susceptible children with the vaccinations of the adults that redirects the spread among them. The fifth hypothesis, which is not self-evident, is that the vaccinations, when done uniformly across age groups above 0-19 age group, have positive feedback on the spread among the age groups over 60. Our basis for this last hypothesis is the difference in mobility and contacts by age group. Preferential vaccination of the age groups with higher contribution to the spread should decrease it to a larger degree than uniformly distributed vaccination if the vaccines slow the spread – if they convey some protection from infection.

Although our modeling is statistical in nature, there are autoregressive components in ARIMA models that should count for the effects of the accumulating number of survivors with their protection against reinfection. Although this number is subject to considerable uncertainty and is limited by our knowledge for the reinfections with SARS-CoV-2, seasonal coronaviruses induce short-lived immunity and we might expect a similar pattern here[18].

The accumulated protective immunity in the form of total cases could be added as a factor, but it has a high correlation with emerging variants. The emergence of new variants is the consequence of that increase in immunity and the co-evolution of humans and the pathogen, seeking equilibrium. This is one reason to expect the role of variants to be harder to detect in our models and to add only them as a factor. They also represent the net effect of acquired immunity. Any detectable influence will be the result of the net balance between increased virulence and immune escape of the viral variants vs the acquired immunity on a population level.

Data and preparation

We use the official weekly date from the Unified National Portal in Bulgaria[19]. New cases are split into age groups - 0-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, 80-89, and 90+ years old. For vaccinations, we use data from [Our World In Data, Coronavirus (COVID-19) Vaccinations, https://ourworldindata.org/covid-vaccinations?country=BGR]. For the dominance of other virus variants, we use data from National Centre for Infectious and Parasitic diseases[20]. Finally, we use the Oxford stringency index data for school closures[21]. The data is categorical – 0-1-2-3 for different levels of school closures.

The data for new cases in different age groups is on a daily basis and is cumulative. We differentiate it and aggregate it weekly with the help of the open-source platform Octave in the interval between June 7, 2020 (week 23) and November 8, 2021 (week 44). We have a set of 74 wkly observations. As can be seen from the data (Figure 1), there is a very clear lag between new cases for 0-19 years, and other age groups – a consecutive movement from lower to higher age groups can be seen before any modeling being done.

The sample crosscorrelation function between new cases for 0-19 year and the MIS-C cases show similar behavior with even larger lags up to 40 d (Figure 2 and 3) which shows the potential usefulness of a model, that describes the pandemic spreads among age groups. Children with MIS-C are lagging further behind the cases among adults.

From the updated Principal Component Analysis (Figure 4), we can see that the 0-19 age groups stand apart from others, and other clear groups are identified – 20-49 years and 60-89 years. For our purposes, here we aggregate 20-49 years and 60-89. We leave 50-59 and 90+ due to a weaker correlation. Our hypothesis for 90+ is that these people live isolated from relatives, often in nursery homes or villages with weak traffic outside of summers, where seasonality decreases the spread. For the 50-59 age group, this is the age group in which children have grown up and are no longer in school. This is one possible explanation for the weaker connection to the 0-19 age group.

We used vaccines and variants data to create factor variables. For vaccines, 0%-9.99% of fully vaccinated people are with value 0%, 10%-19.99% are with 1%, and 20%-29.99% are with 2. For variants, the wild type from Wuhan is with 0; alpha is with 1, and Delta is with 2. Therefore, we do not consider it, for now, Delta+ separately, but when it gets dominant, we can add another value, 3, and we will update our model. As we showed in our previous research on which we step here, there are serious correlations between new cases in different age groups, which is the reason why we aggregate cases by combining age groups.

Factors contributing to the spread of COVID-19 among children

First, we investigated the spread among children. To test it, we used regression with ARIMA errors. Like in our previous research, we use R, with packages "forecast" and "urca". We examine for stationarity the time series with two different tests - Kwiatkowski-Phillips-Schmidt-Shin (KPSS) tests[22] and the Augmented Dickey-Fuller test (ADF)[23].

In the case of weekly data for 0-19 years old, both tests gave an order of differentiation 1. Therefore, we used the ARIMA model with fixed order of differentiation 1 and the auto for our purposes.arima() function[24] to select the best possible model. Our regressors are the variants factor and the vaccines factor. In addition, the same function estimates the dynamic regressions by adding the predictors thanks to the argument "xreg." After some additional lag selection of the chosen regressors, based on RMSE, MAPE, MAE, AIC, practical considerations and the standard errors of the coefficients, we obtained an optimal model - Model I (Tables 1 and 2, Figures 5 and 6) based on minimal data errors covering the period from June 7, 2020, to November 2, 2021.

Temporal spread across age groups

One of our significant hypotheses is the spread across age groups – from children to parents and grandparents in households and by secondary contacts. There are clear lags, shown in Figure 1, for which we model here. We separate the other age groups into two main groups – the group of parents 20-49 and the group of grandparents with contacts to children 60-89. The age group 50-59 is largely devoid of direct contact with children or grandchildren, and the same is for 90+. We used second-order

Table 1 Correlations between the variables for Model I – factors contributing to the spread among children

Variables	Variants	Vaccines	Schools L1	Schools L2
Variants	1	0.7877448	-0.4553950	-0.4553950
Vaccines	0.7877448	1	-0.4089070	-0.4502049
Schools L1	0.4553950	-0.4089070	1	-0.4089070
Schools L2	0.4553950	0.4502049	0.8506835	1

Table 2 Regression models with ARIMA (0,1,1) errors - Model I – factors contributing to spread among children

Model summary		
Coefficient	**Estimate**	**Standard error**
MA1	0.4995	0.1272
Variants[a]	71.0883	98.4872
Vaccines L2	**191.2172**	114.5689
Schools L1	**-103.5798**	57.1372
Schools L2	-37.4081	56.7021
R^2	**0.926**	
RMSE	**154.84**	
Bias	**8.79**	
MAPE	**43.35**	

[a]This variable is not so well estimated because the newest variant has not enough data.

differencing on our model to account for quadratic growth[25] and decrease the correlation between predictors to acceptable levels. Using the similar criteria as in Model I, we receive optimal model - Model II (Tables 3 and 4, Figures 7 and 8)

For our third model – Model III, we use the same regressors, but this time against the new weekly cases for the age groups 20-49 (Table 5, Figures 9 and 10).

Epidemiology of MIS-C in Pirogov: Characteristics of MIS-C dynamics and modeling approach

On the individual level, the onset of symptoms from MIS-C is, on average, 5 wk (4 to 6 wk) after the acute phase of the disease[26]. On the regional level, however, we see very clearly much more significant delays from cross-correlation analysis between new cases of 0-19 years old and new cases of MIS-C on a weekly basis (Figures 2 and 3). The delays are 41-48 wk between new cases and MIS-C cases. One possible explanation for this is that MIS-C is a very low probability event, which means that many covid-19 cases are necessary for one mis-c case to happen, and a lot of time must pass to accumulate these cases. Another possible explanation is that the analysis included data from a single center in the capital of Bulgaria, which cannot be fully representative considering that the Department is intensive and urgent but has no cardiology profile.

On the other hand, accumulating data for more than one year can make it possible to search, including the "seasonality" hypothesis. However, when December came, naturally, there was a peak again. Unfortunately, however, we did not find a description of this phenomenon in the literature.

"In this cohort study of 248 persons with MIS-C, MIS-C incidence was 5.1 persons per 1000000 person-months and 316 persons per 1000000 SARS-CoV-2 infections in persons younger than 21 years. Incidence was higher among Black, Hispanic or Latino, and Asian or Pacific Islander persons compared with White persons and in younger persons compared with older persons."[27]. Another more straightforward but less likely explanation is due to the dynamics of repeated waves which are highly correlated with each other (very similar in nature). It is less likely due to the significantly higher correlations between cases and MIS-C for the more considerable lags of 41-48 wk. Whatever the cause, a simple predictive model benefits from the lags with higher correlations.

The epidemiological data includes 4 additional MIS-C cases, a total of 39, which arrived in weeks 77th and 78th since June 6, 2020 (the initial date for new cases by age groups time series), which is November 29, 2021 - December 5, 2021. The relative growth does not display these leading delays (lags) of over 40 wk, which shows why it was necessary to compute the cross-correlation (Figure 2) function in the first place. The ADF shows one root at unity – the process of MIS-C arrival is non-stationary, but its first

Table 3 Correlations between the variables for Model II

Variables	Variants	Vaccines	0-19 L1	0-19 L2	0-19 L3
Variants	1	0.00	-0.08	0	0.00
Vaccines	0	1	-0.19	-0.13	0.34
0-19 L1	-0.08	-0.19	1	-0.40	-0.04
0-19 L2	0	-0.13	-0.40	1	-0.28
0-19 L3	0.00	0.34	-0.04	-0.28	1

Table 4 Regression models with ARIMA (0,2,1) errors - Model II

Model summary		
Coefficient	**Estimate**	**Standard error**
MA1	-0.4661	0.1438
Variants[a]	147.7111	385.2214
Vaccines	**548.6656**	412.7460
0-19 L1	1.2614	0.5521
0-19 L2	**1.4164**	0.5521
0-19 L3	1.1012	0.6543
R^2	**0.962**	
RMSE	**620.16**	
Bias	**-16.44**	
MAPE	**23.38**	

[a]This variable is not so well estimated because the newest variant has not enough data.

Table 5 Regression models with ARIMA (0,2,1) errors – Model III

Model summary		
Coefficient	**Estimate**	**Standard error**
MA1	-0.9502	0.1272
Variants[a]	724.0295	641.8759
Vaccines	**1095.1284**	691.3952
0-19 L1	**5.7357**	0.0519
0-19 L3	1.9966	0.8769
R^2	**0.953**	
RMSE	**897.5266**	
Bias	**-72.74389**	
MAPE	**31.96102**	

[a]This variable is not so well estimated because the newest variant has not enough data.

finite difference is a stationary process. Therefore, at least one differencing is needed for Arima modeling.

Probabilistic modeling of MIS-C dynamics

We can consider the arrival times of children with MIS-C as independent in time. This is possible due to the perceived random nature of the occurrence of MIS-C due to independent factors and the lack of

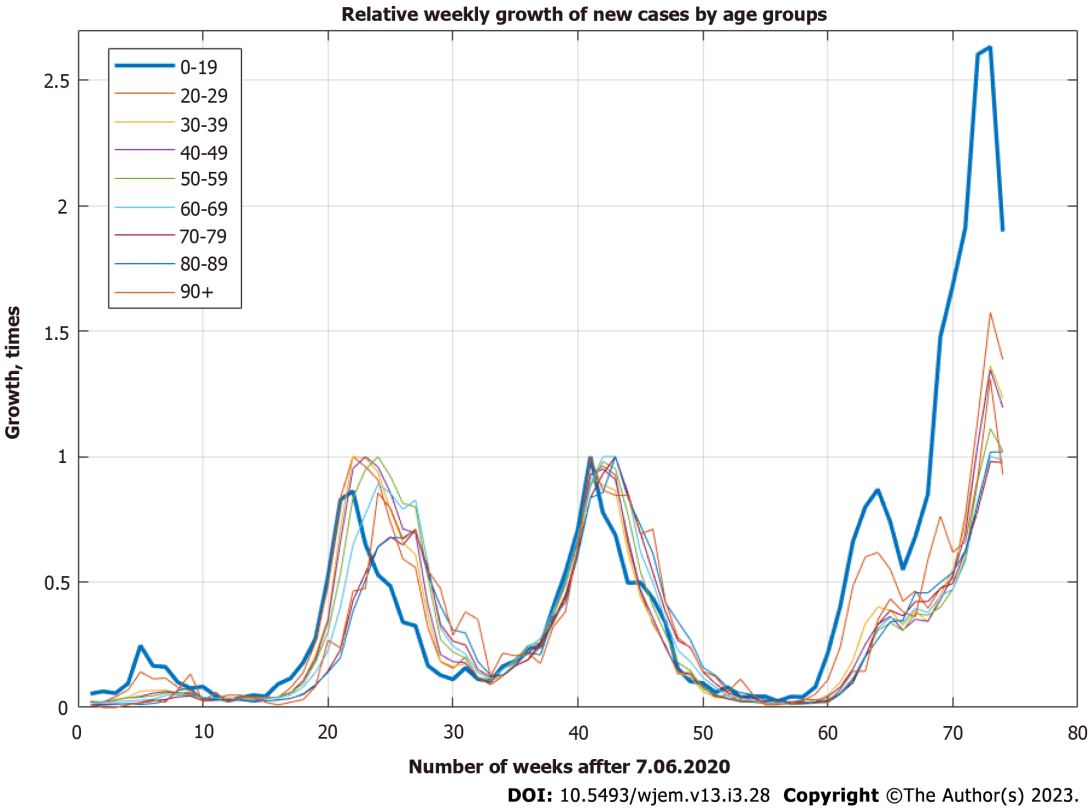


Figure 1 Relative growth of cases by age groups. An apparent lag between 0-19 and others can be seen.

clear predictors of which child will have MIS-C in the post-acute phase. Therefore, the most suitable distribution for independent arrivals is the Poisson distribution.

We have to note that this modeling is most appropriate when the mean value is constant, which is not so during the pandemic, in which the process of MIS-C arrivals is non-stationary. In a post hoc analysis, this Poisson distribution may change its lambda. However, it can still be used to forecast by taking the λ as a parameter in time λ (t) and forecasting its change. Then at every point in time, this distribution can be recalculated, and we can have the expected value and some confidence interval for which the number of arrivals at a given time period will be there with predefined probability (for example, 95% confidence interval).

Time series analysis

To obtain a model that explains the variation relatively well for this small set of data (39 cases), we grouped both new cases for 0-19 on a national level and MIS-C in one center –the Pediatric department and Pediatric ICU in the main Emergency Hospital in the capital city on a bi-weekly basis. This allowed us to have a good evaluation of the NC L21 parameter (Table 6), which shows the new cases 21 bi-weekly periods ago.

In general, the MIS-C cases in Pirogov are 1% of the cases among 0-19, which gives roughly 2%-3% in the respective age group for that disease, which is a subset of 0-19. This is incredibly high compared to the relatively low frequency of MIS-C, as we quoted already. Nevertheless, this can be used to obtain a very rough estimation for the number of children missed by testing if we combine this set with the data from other hospitals in Bulgaria.

RESULTS

Model I - factors contributing to the spread of COVID-19 among children

The optimal model is Arima (0,1,1) (Table 2, Figures 5 and 6) and uses school closures with lags of 1 and 2 wk, variants with lag 0 wk and vaccines with a lag of 2 wk. We ensure that the correlation between the four predictors (regressors) is acceptable, as seen in Table 1. The model summary is given in Table II. The residuals are shown in Figure 5, and the model fit – is in Figure 6. A rule of thumb for a good fit is to have an absolute value of a coefficient twice as significant as its standard error.

As Table 2 shows, school closures and vaccines have significant impacts on the spread among children – 0-19 years old age group. Michos *et al*[28] reported the hospitalization rates of children due to

Table 6 Regression models with ARIMA (0,1,0) errors

Model summary		
Coefficient	**Estimate**	**Standard error**
NC L21	**0.0016**	0.0008
NC L22	-0.0011	0.0007
NC L23	1e-04	7e-04
R^2	**N/A**	
RMSE	**0.8425338**	
Bias	**N/A**	
MASE	**0.4601526**	

N/A: Not applicable.

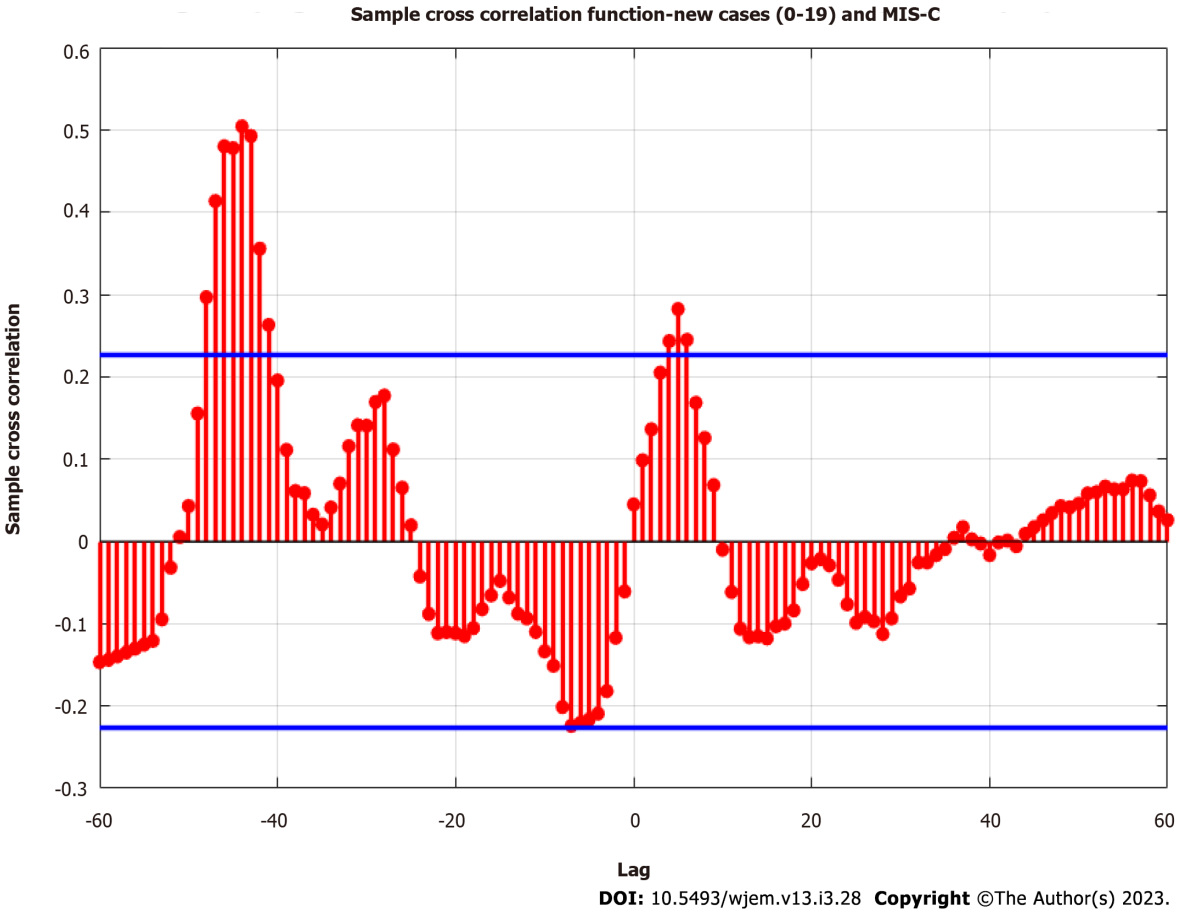


Figure 2 Sample cross-correlation function between new weekly cases of coronavirus disease 2019 and multisystem inflammation in children cases. MIS-C: Multisystem inflammation in children.

COVID-19 in Greece and the United States[29,30].

In terms of age groups, among > 1.2 million children below 18 years of age with SARS-CoV-2 infection in the USA between March and December 2020, children were distributed as follows: Preschool (age 0 through 4 years)–7.4%; elementary school (age 5 through 10 years)–10.9%; middle school (age 11 through 13 years)–7.9%; high school (age 14 through 17 years)–16.3%. However, in Bulgaria, no such information is available.

Closures decrease significantly the numbers – closure of level 3 falls on average with 310 cases per week or a minimum of 30% reduction of the number of cases. It is a well-estimated coefficient with a standard error of half its value. Close to that proper precision of estimation is the Vaccines factor, which gives 192 cases weekly per 10% of fully vaccinated adults or an overall close to 400 cases per week

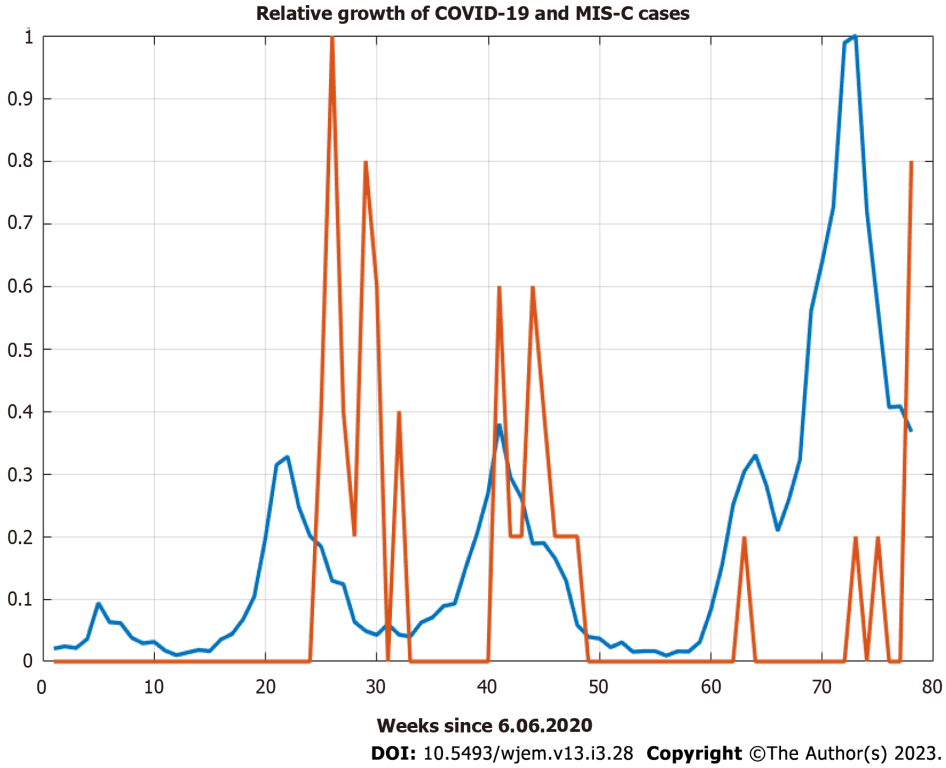


Figure 3 Relative growth of national-level coronavirus disease 2019 cases and multisystem inflammation in children cases in Pirogov Hospital. COVID-19: Coronavirus disease 2019; MIS-C: Multisystem inflammation in children.

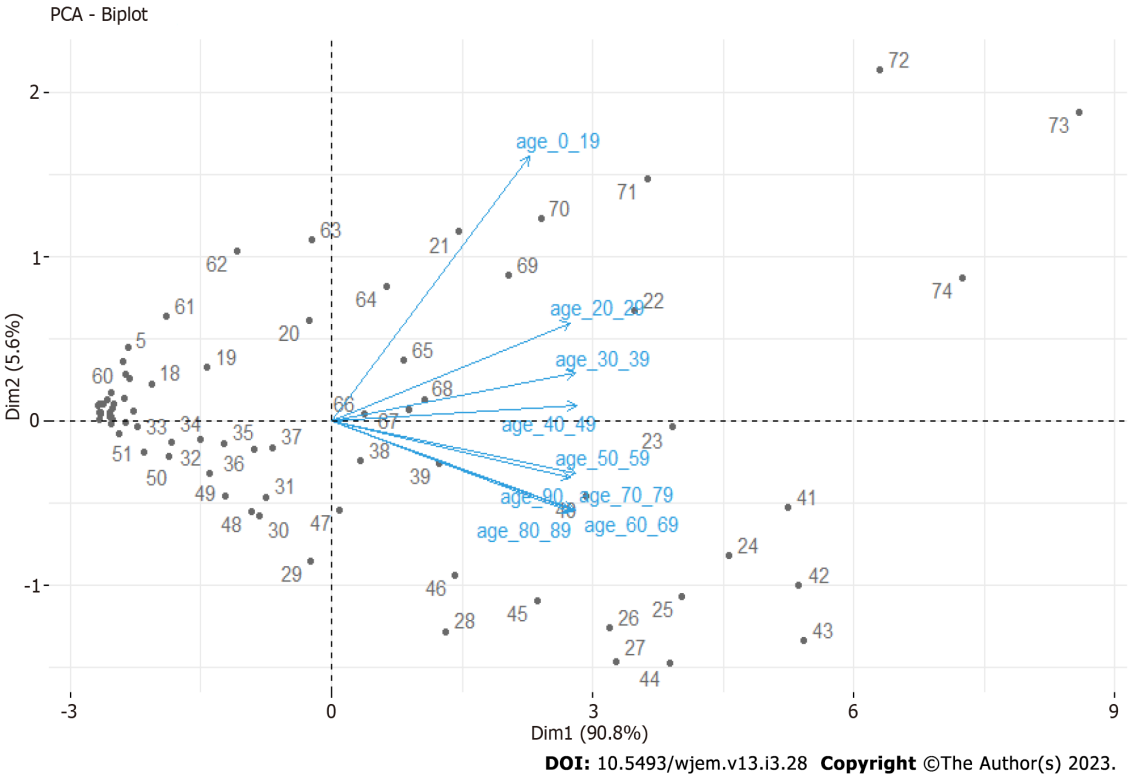
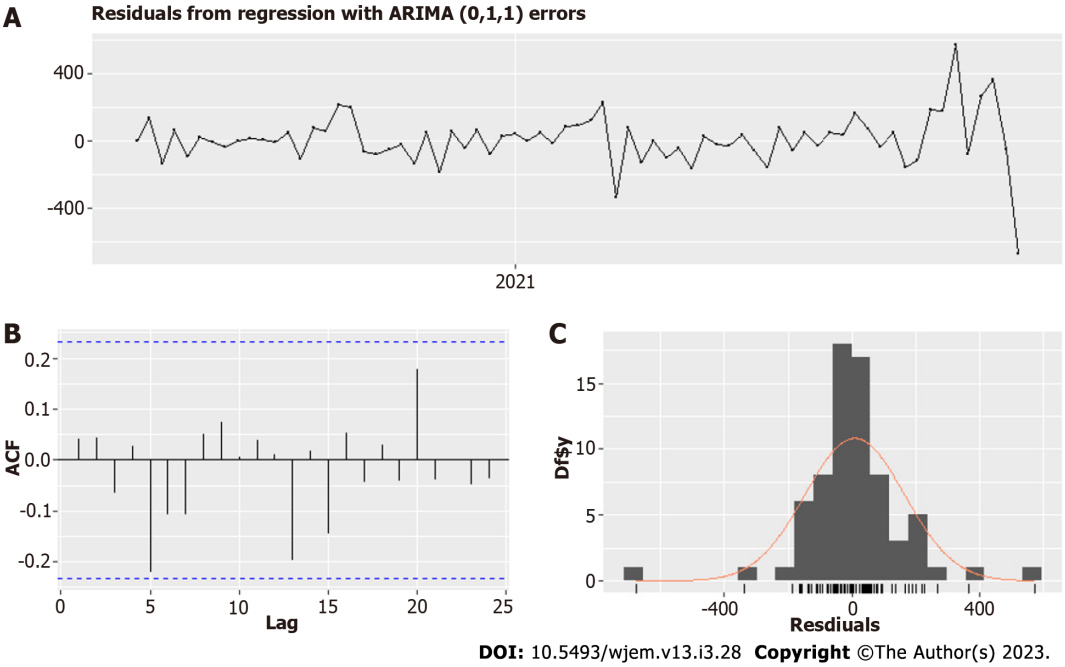


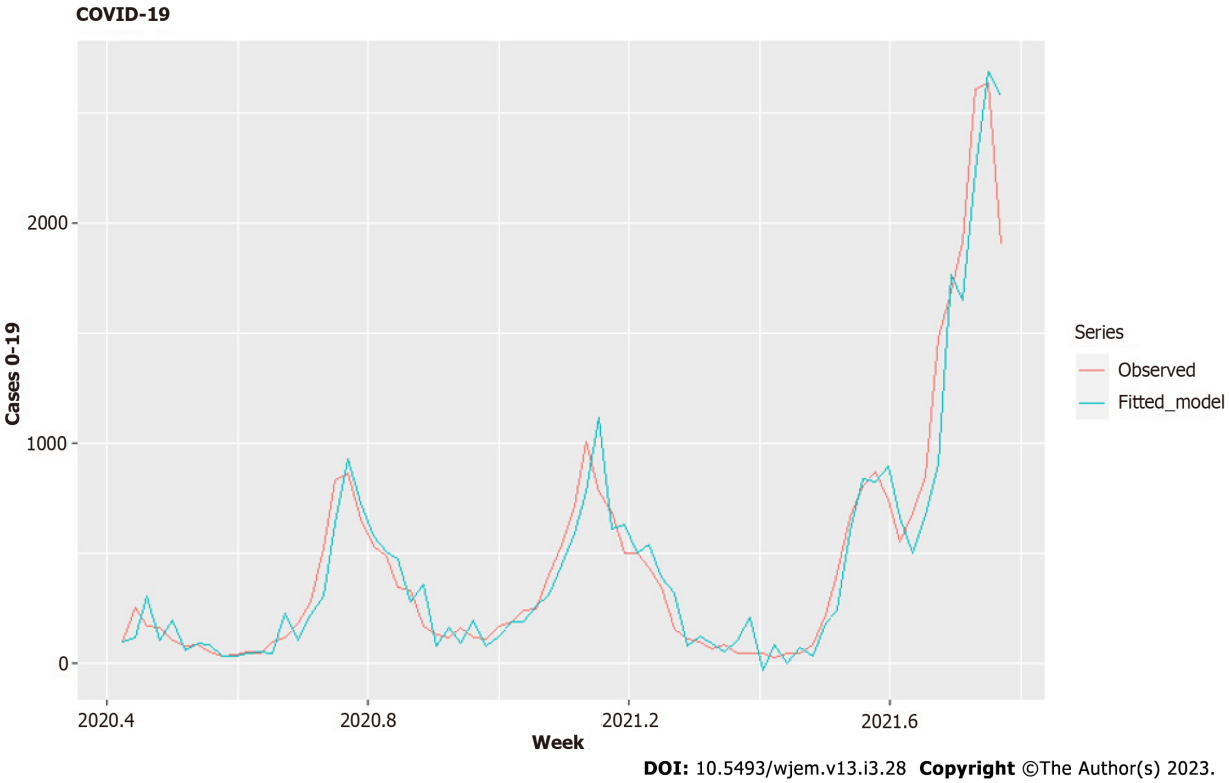
Figure 4 Principal component analysis Biplot for age groups on a weekly basis. PCA: Principal component analysis.

increase. We see here that this is a probable conclusion – vaccinations of adults accelerate the spread among mostly unvaccinated children.



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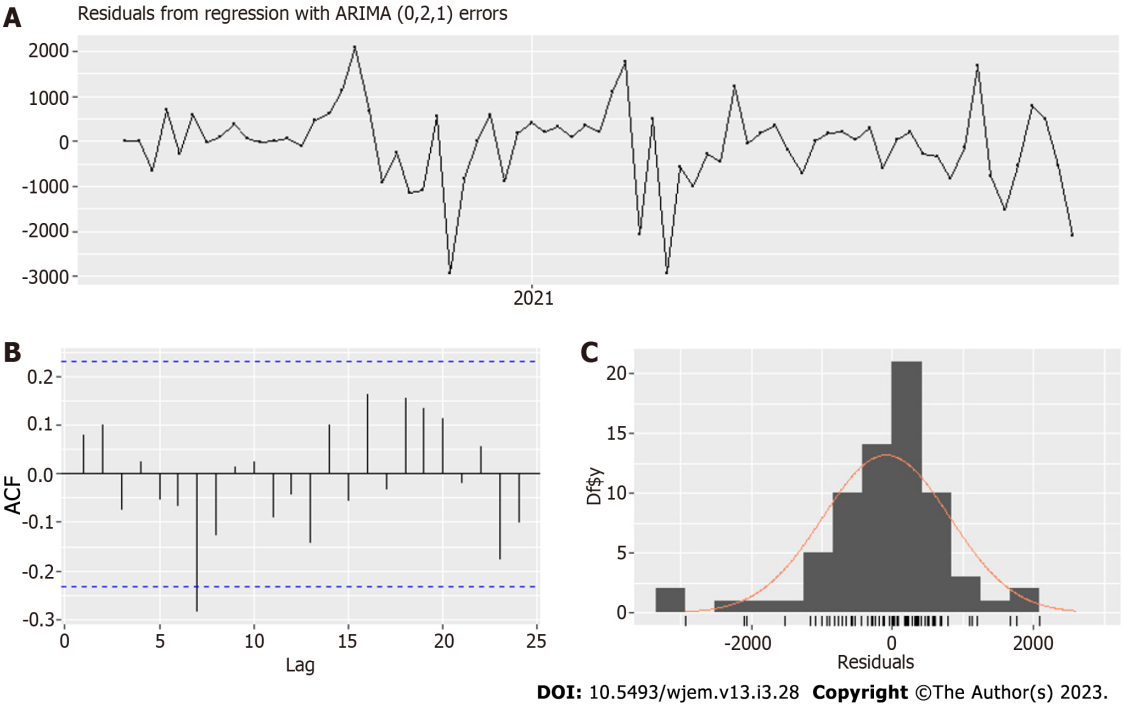
Figure 5 Residuals of Model I – Factors contributing to the spread among children. A: Residuals of the model; B: Autocorrelation function of the residuals; C: Distribution of the residuals.



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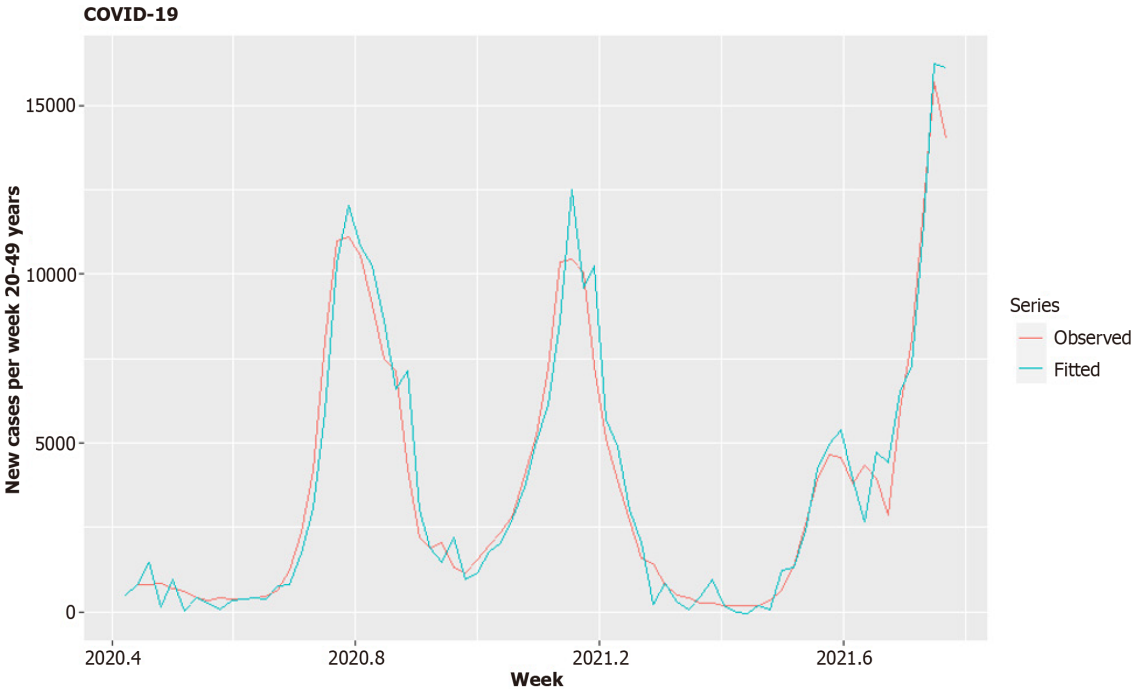
Figure 6 Fitted Model I – Factors contributing to the spread among children. COVID-19: Coronavirus disease 2019.

Despite all issues with data related to the testing of children in Bulgaria, we managed to explain 93% of the variation with our model. With this currently limited data, the role of variants remains unverified due to a relatively high correlation with vaccinations – they have similar dynamics. There is also an accumulation of immunity through prior infection that competes with the variants' tendency to increase rates among children. We may update or change the model accordingly after the Delta and Omicron wave to include more values to the variants factor and to check whether variants accelerate the spread or distinguish it from the vaccination pressure.



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Figure 7 Residuals of MODEL III –III – Temporal spread across age groups 20-49. A: Residuals of the model; B: Autocorrelation function of the residuals; C: Distribution of the residuals.



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Figure 8 Fitted MODEL III – Temporal spread across age groups 20-49. COVID-19: Coronavirus disease 2019.

Models II and III - temporal spread across age groups

For Model II and the age group of 60-89, the correlations (Table 3) are acceptable, and most of the coefficients are well estimated with small standard error, excluding variants (Table 4). The residuals are shown in Figure 7. The cases in the 0-19 group explain 96.2% of the variation in cases for the 60-89 years group (Figure 8).

The results for Model III showed that the new cases among 0-19 years old with a lag of two weeks are not necessary to predict the dynamics of the age group 20-49, and its removal improved the model. The correlations of this subset of regressors are the same as in Table 2. The model summary is given in

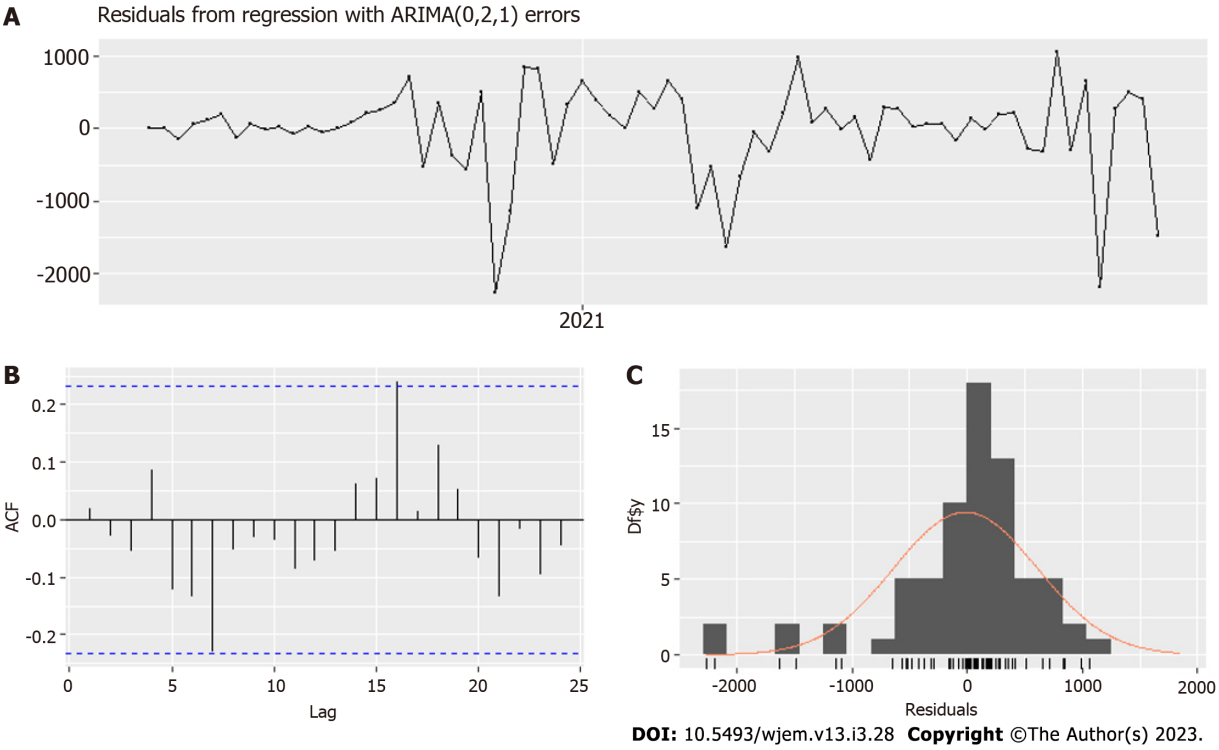


Figure 9 Residuals of Model II – Temporal spread across age groups 60-89. A: Residuals of the model; B: Autocorrelation function of the residuals; C: Distribution of the residuals.

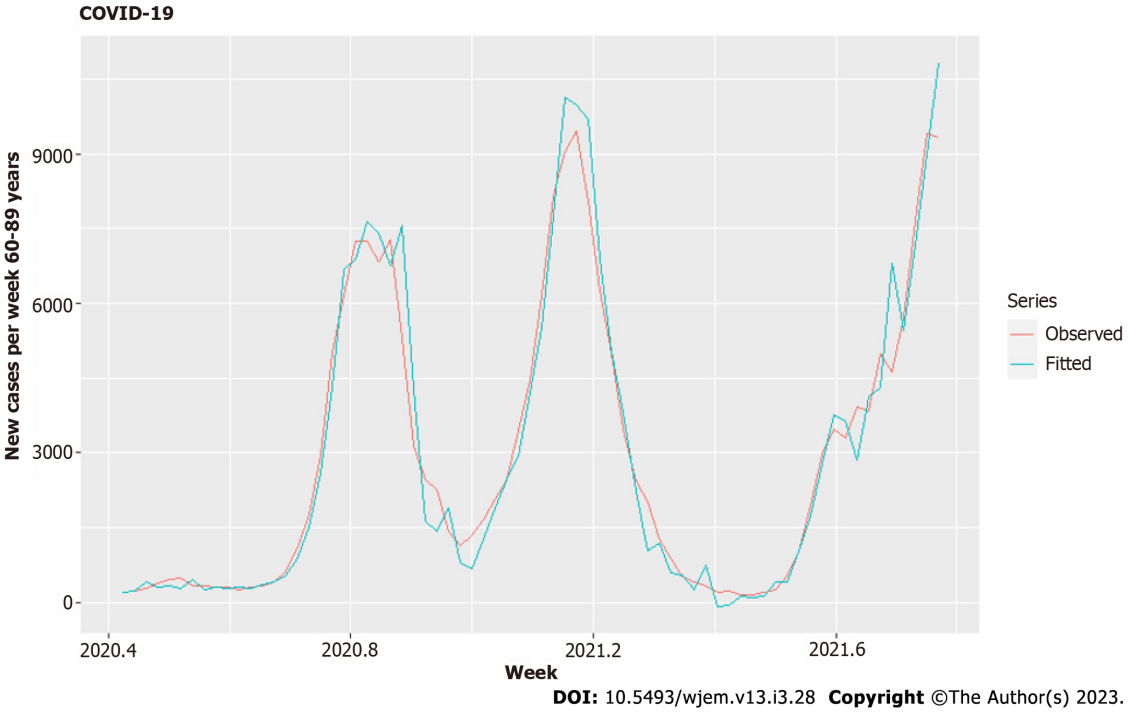


Figure 10 Fitted Model II – Temporal spread across age groups 60-89. COVID-19: Coronavirus disease 2019.

Table 5, the residuals are in Figure 9, and the fitted model is in Figure 10.

For the age group of 20-49 years, the influence of vaccinations in accelerating the spread among them is twice as strong – the coefficient in Table 5 is double relative to the same coefficient in Table 4 and with a relatively smaller standard error of estimation, bordering the proper ratio of 2:1 coefficient to error. One part of the explanation is that they are closely linked to children – the coefficient with Lag 1, which corresponds to the household infections is 5 times larger (a household effective reproductive number of 5.73 and the standard error of estimation is ten times smaller, than the error of the same coefficient in

the model for 60-80. The ratio of coefficient-to-standard error here jumps from 2:1 to 100:1. The role of the variant becomes probable in this age group – now, the standard error of estimation is smaller than the estimated coefficient. The current insufficient set of 74 observations increases the likelihood of coming to a positive conclusion for variants and spread among young people after the pandemic despite the acquired immunity of prior infections. Such a conclusion is also possible for the 0-19 age group, with a standard error of estimation close to the value of the coefficient, but is less likely for people in the 60-89 age group. It appears that the acceleration in the spread from variants is age dependent. One possible explanation is that with the increase of R0 of the variants, the age groups with more intensive contacts get more "advantage" over other age groups. Another explanation would be a saturation point in age risks from the pathogen – a weaker immune system that lowers the threshold for the effects of virulence (and R0) on the spread. This would mean that from some R0 and above, the spread cannot accelerate further for specific age groups. These hypotheses need further analysis and testing in future research.

Results for MIS-C dynamics and probabilistic modeling

Probabilistic modeling is a less precise way to predict or explain the dynamics than the time series analysis approach we employed in this article's first section. The distribution in Figure 11 shows us there is over a 5% chance of having 5 children per week, which gives a wide interval of new beds are needed weekly, and with the non-stationarity of the process, this has to be recalculated every week. With the increase in prevalence among children with new viral variants, it is possible $\lambda(t)$ to increase further, which means that we need to forecast it to do these recalculations. The estimating procedure will amplify the forecast error (fitting data to the Poisson distribution). Therefore, we prefer the time series analysis approach even for the small number of MIS-C cases.

Results for time series analysis of MIS-C cases

Results for MIS-C are promising but not yet conclusive. We need larger datasets to which to apply our methodology to have a better prediction on a weekly basis. We consider this an extreme test of our approach – can we model the MIS-C dynamics from such a small set? It appears that we can. In our model, we try to regress the new MIS-C cases against the new cases for the age group 0-19 years (we don't have detailed data by smaller sub-groups for the entire pandemic).

Our model in Table 6 is for biweekly cases. Coefficient NC L21, which is with a lag of one biweekly period, or up to 14 d, is well estimated with a value to standard error 2:1. We have close to good evaluation for NCL22 (which is with a lag of two periods, or up to 4 wk) with ratio 1.58. For a good assessment of NC L23, we need more data. The significant lags allow us to model properly only after half of the period of 78 wk, so we consider these results as promising, not conclusive. However, even with that small data set, we can give some predictions with Model IV (Figures 12 and 13) and some explanation of the data variation. We will continue the effort to model these cases by expanding our dataset in joint research with other hospitals in Bulgaria.

DISCUSSION

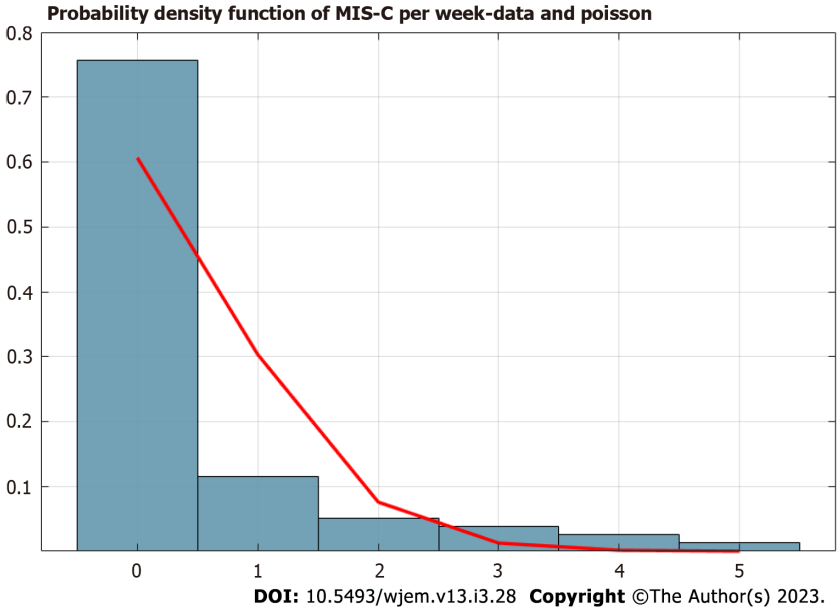
We aimed to assess the role of children in the ongoing epidemic in Bulgaria and to predict the impact of the pandemic on MIS-C cases in Pirogov. The initial claims in the literature that children have no impact and schools do not need strict measures do not hold in our analysis. Furthermore, it is clear that children are drivers of the epidemic in Bulgaria, probably due to the few school measures and no quarantines for their families when a class is quarantined[31].

The role of children in the Bulgarian COVID-19

We were able to predict cases in two age groups that are societally linked to children – 20-49 and 60-89 – parent and grandparents (and their close same-age contacts). Even before modeling, a large lag between cases in children (0-19) and cases in these groups was visible (Figure 1). What brings us more information is the difference between these two groups and the age groups that are less likely to be connected in the same household with children – 50-59 and 90+. As our analysis shows, school closures were a vital part of the containment of previous waves[32,33]. For that reason, not only children with their intense contacts but children *via* schools were drivers of the pandemic waves up to and including Delta[34]. Although there are summer waves from children to adults (2020 and 2021), they are transient and reflect migrations from cities to villages and tourist destinations, while autumn and winter waves show stable trajectories while schools are opened and turn after the closures – which our model I captured.

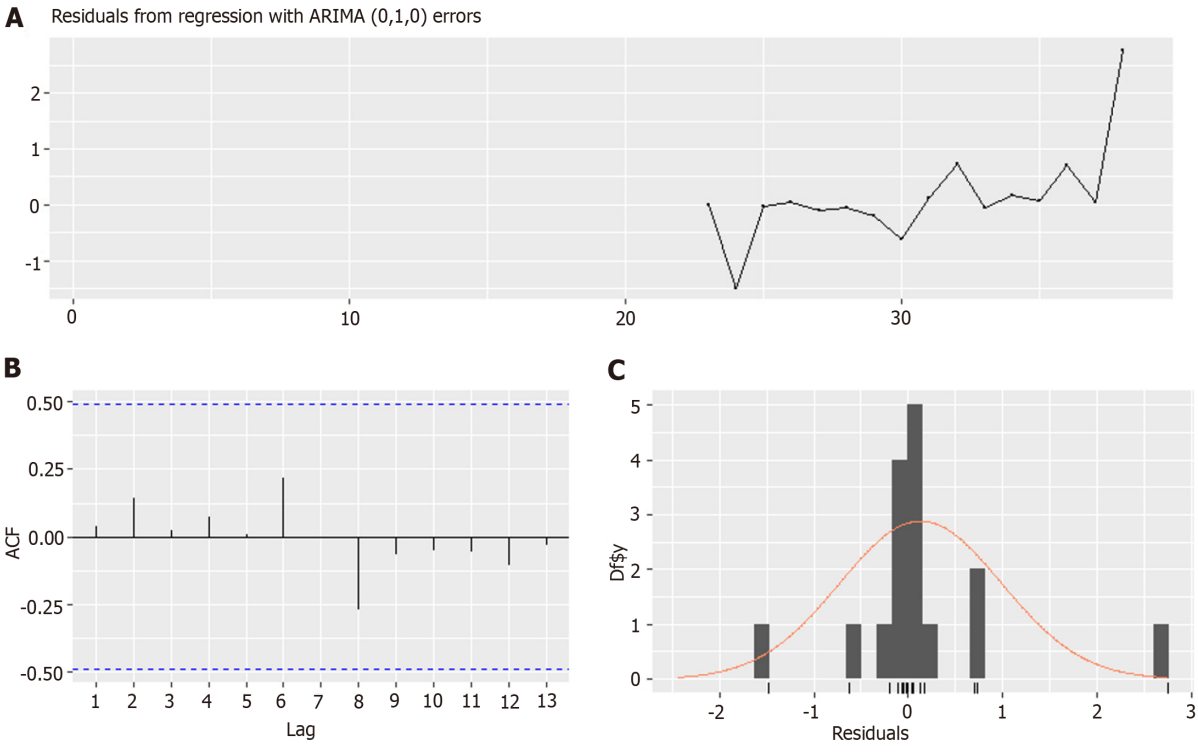
The counterintuitive role of vaccinations

We can see some plausible counterintuitive influences of vaccinations on the spread among adults in 60-89 years. It appears to have a strong positive impact, accelerating the spread (of course, for such conclusions, more research is necessary). The standard error of estimation is smaller than the coefficient. It is not small enough to accept the value of the coefficient but enough to give us some confidence about



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Figure 11 Probability density distribution of the number of multisystem inflammation in children cases on a weekly basis. Poisson distribution for total cases up to 4.12.2021 is with λ = 0.50∈ [0.355, 0.835]. MIS-C: Multisystem inflammation in children.



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Figure 12 Residuals of model for the bi-weekly period. A: Residuals of the model; B: Autocorrelation function of the residuals; C: Distribution of the residuals.

the direction of the influence. The current vaccination policy in Bulgaria, which is not focused on risk groups, does more harm than good since the mortality risk grows exponentially with age[16]. What is the reason? At first glance, the paradoxical negative impact of the vaccination program on increasing the incidence of COVID-19 can be explained by the rapid relaxation of anti-epidemiological measures at a deficit of vaccination coverage. The delay according to the model of the MIS-C case by 1 year is interesting because right now we have such a wave, one year after the previous one."

Furthermore, vaccines accelerate the spread significantly among children, as our first model showed, and as our second model demonstrates, the spread among 60-89 is strongly predicted with lags of 1, 2

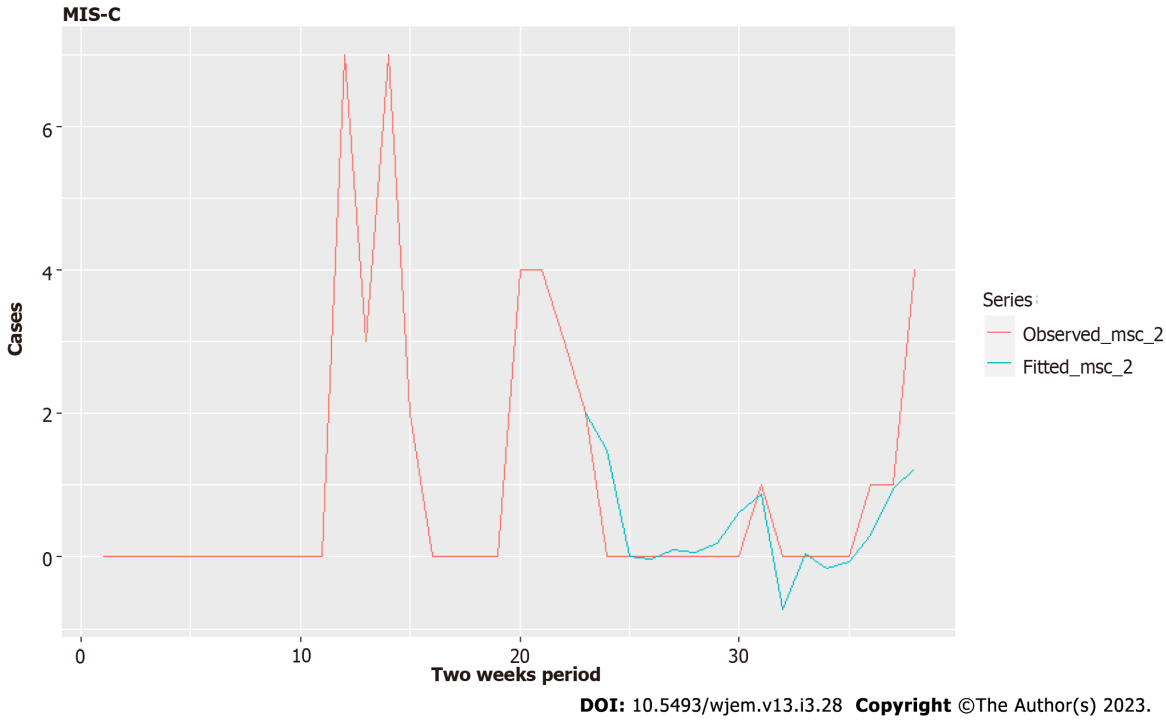


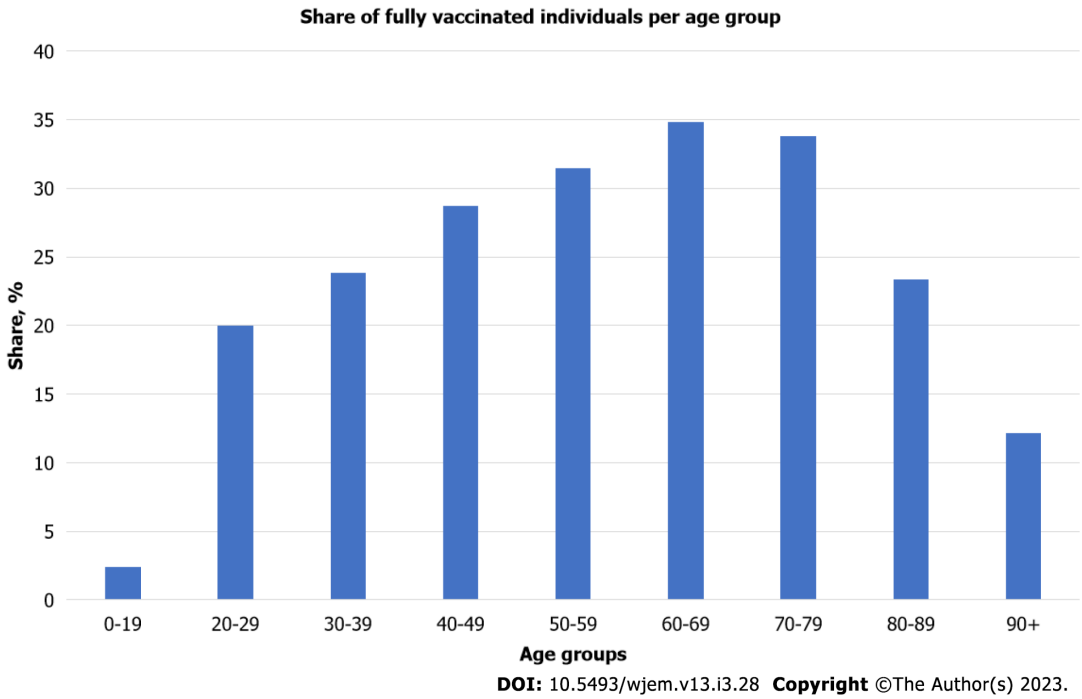
Figure 13 Fitted model for a bi-weekly period. MIS-C: Multisystem inflammation in children.

and 3 wk from cases in the 0-19 age group. The lack of exponential distribution of vaccinations across age groups (Figure 14) that would match the risk probably leads to a net negative effect on the number of cases among older individuals. Currently, the low vaccination rate translates this negative effect into a negative impact on deaths, which can be estimated from our model for the age-mortality risk. This might change with an increase in vaccination coverage or a change in the distribution of vaccination coverage.

CONCLUSION

Our modeling and data show with high probability that in Bulgaria, with our current measures, vaccination strategy and contact structure, the pandemic is driven by the children and their contacts in school. This is the simplest explanation of our three models. Furthermore, the lack of vaccination strategy accelerates the spread among children and from them to the age groups linked to them. For a country with low vaccination coverage of 23% up to November 16, 2021, this translates into an increased number of deaths and hospitalizations. There is a possibility of emerging variants to accelerate their spread among people below 50 years, thus compensating for the effect of acquired partial herd immunity, but that has to be confirmed by a larger set of observations and possibly new models to catch precisely the effects of the variants. This hypothesis and the other two we outlined as reasons for this age-restricted acceleration of the spread are part of our future research, as long as updating these three models after the end of the delta wave.

The modeling of MIS-C cases comes with few surprises – first, it is possible to model it with ARIMA even for a very low case amount, only for Pirogov, not on a national level. Suppose we put together all cases in Bulgaria. In that case, we will have a substantially larger dataset and the ability to make practically usable forecasts for MIS-C based on the historical record. Second, the optimal lags for prediction are huge – 41 to 48 wk or 21 to 24 bi-weekly periods. This is due to the low probability of occurrence of MIS-C among children or data artifacts (in which case it will change with a larger dataset). Third, the Poisson distribution can be used for modeling the probability of n cases arriving at a given week, but this is not a surprise, considering the independence of cases from one another in time. A challenge for modeling will be the arrival of new variants such as Delta, Delta+ and Omicron, which can change this probability and make historical data less useful for prediction. For that reason, we need the full dataset of MIS-C to model the dependency between the cases and the variants, which is part of our plan for future research, as well as modeling the network of covid-19 spread across ages in more detail, with more data for 0-19 subgroups (it appeared for first time in 25.09.2021 in the open data portal[35,36] and more elaborate dynamic network models).



DOI: 10.5493/wjem.v13.i3.28 **Copyright** ©The Author(s) 2023.

Figure 14 Share of fully vaccinated individuals per age group up to November 16, 2021. Data for the number of fully vaccinated individuals is taken from[35].

ARTICLE HIGHLIGHTS

Research background

Transmission from children to adults was deemed secondary to the transmission from adults to children. After the first big wave in September to December 2020, masks were mandated in grades 5 and above but not enforced, with arguably low adherence, less than the general adherence due to mask removal in breaks between classes.

Research motivation

With starting the pandemic in Bulgaria, children were mostly speared from infection. On the one hand, it was thought that coronavirus disease 2019 (COVID-19) took a mild course in children. On the other hand, the testing was at the lowest rate among children. Up to now, the total number of infected children (0-18 years) is 103743.

Research objectives

We aimed to demonstrate the role of children in the COVID-19 spread in Bulgaria and to test the hypothesis that there are no secondary transmissions in schools and from children to adults.

Research methods

We used ARIMA models with external regressors and Poisson distribution modeling to test our hypotheses.

Research results

Our models allows to predict the new weekly cases in different age groups from the new cases in children one and two week prior. The age groups 50-59 and 90+ are less predictable and are also more isolated from children, consistent with the idea that children drive the pandemic in Bulgaria.

Research conclusions

Our models support the hypothesis that children are drivers of the pandemic in Bulgaria.

Research perspectives

In the first year of the pandemic it was suggested that children rarely transmit the virus to adults. On this basis Bulgarian Ministry of Health decided to allow the parents of quarantined children to continue to work and move freely in society. We will use more sophisticated models such as branching process to demonstrate that even if the probability of infection from children to adults is very low, the free

movement of the parents will still trigger a pandemic wave.

FOOTNOTES

Author contributions: Tomov L and Velikova T contributed to conceptualization; Tomov L, Lazova S, Batselova H, Ganev B, Tzocheva I, and Velikova T contributed to resources and literature review; Tomov L, Lazova S, Batselova H, Ganev B, and Tzocheva I contributed to writing – original draft preparation; Velikova T, Ganev L contributed to writing – review & editing; Velikova T contributed to supervision; All authors revised and approved the final version of the manuscript.

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Country/Territory of origin: Bulgaria

ORCID number: Latchezar Tomov 0000-0003-1902-6473; Hristiana Batselova 0000-0002-6201-848X; Snezhina Lazova 0000-0002-5884-7760; Borislav Ganev 0000-0001-9699-4983; Iren Tzocheva 0000-0001-9777-5942; Tsvetelina Velikova 0000-0002-0593-1272.

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REFERENCES

1 **Kendall EK**, Olaker VR, Kaelber DC, Xu R, Davis PB. Association of SARS-CoV-2 Infection With New-Onset Type 1 Diabetes Among Pediatric Patients From 2020 to 2021. *JAMA Netw Open* 2022; **5**: e2233014 [PMID: 36149658 DOI: 10.1001/jamanetworkopen.2022.33014]

2 **Heiss R**, Tan L, Schmidt S, Regensburger AP, Ewert F, Mammadova D, Buehler A, Vogel-Claussen J, Voskrebenzev A, Rauh M, Rompel O, Nagel AM, Lévy S, Bickelhaupt S, May MS, Uder M, Metzler M, Trollmann R, Woelfle J, Wagner AL, Knieling F. Pulmonary Dysfunction after Pediatric COVID-19. *Radiology* 2023; **306**: e221250 [PMID: 36125379 DOI: 10.1148/radiol.221250]

3 **National Institutes of Health**. Accessed [12-18-2022]. Available from: https://www.covid19treatmentguidelines.nih.gov/

4 **Lazova S**, Alexandrova T, Gorelyova-Stefanova N, Atanasov K, Tzotcheva I, Velikova T. Liver Involvement in Children with COVID-19 and Multisystem Inflammatory Syndrome: A Single-Center Bulgarian Observational Study. *Microorganisms* 2021; **9** [PMID: 34576853 DOI: 10.3390/microorganisms9091958]

5 **Atanasov K**, Gorelyova-Stefanova N, Tsocheva I, Psederski Hr, Lazova Sn. Disease spectrum in children with multisystemic inflammatory syndrome (MIS-C) - clinical case, Emergency Medicine, 2021. Last access on May 16, 2023. Available from: https://pirogov.eu/bg/download/2419

6 **Ganev B**, Lazova S, Genova K, Tzotcheva I. Clinical variants of cardiovascular involvement in sars-cov-2 associated multisystem inflammatory syndrome in children - a case series, electronic presentation, XV National Pediatric Congress, Borovets, 2021

7 **Dasheva A**, Telcharova A, Ganeva M, Stefanov S, Vasileva Z, Nenova K, Kaneva A, Genova K. Multisystemic inflammatory syndrome in children and acute myocarditis after SARS-CoV-2 - description of 6 cases and literature review, Pediatria, issue 3, 2021

8 **Rakhsha A**, Azghandi S, Taghizadeh-Hesary F. COVID-19 pandemic and patients with cancer: The protocol of a Clinical Oncology center in Tehran, Iran. *Rep Pract Oncol Radiother* 2020; **25**: 765-767 [PMID: 32765192 DOI: 10.1016/j.rpor.2020.07.001]

9 **Rakhsha A**, Azghandi S, Taghizadeh-Hesary F. Decision on Chemotherapy Amidst COVID-19 Pandemic: a Review and a Practical Approach from Iran. *Infect Chemother* 2020; **52**: 496-502 [PMID: 33263246 DOI: 10.3947/ic.2020.52.4.496]

10 **Li X**, Wang W, Yan S, Zhao W, Xiong H, Bao C, Chen J, Yue Y, Su Y, Zhang C. Drug-induced liver injury in COVID-19 treatment: Incidence, mechanisms and clinical management. *Front Pharmacol* 2022; **13**: 1019487 [PMID: 36518661 DOI: 10.3389/fphar.2022.1019487]

11 **Heavey L**, Casey G, Kelly C, Kelly D, McDarby G. No evidence of secondary transmission of COVID-19 from children attending school in Ireland, 2020. *Euro Surveill* 2020; **25** [PMID: 32489179 DOI: 10.2807/1560-7917.ES.2020.25.21.2000903]

12 **Tang JW**. SARS-CoV-2 and aerosols-Arguing over the evidence. *J Virol Methods* 2021; **289**: 114033 [PMID: 33285192 DOI: 10.1016/j.jviromet.2020.114033]

13 Ministry of Education and Science, Order RD09-3310/ November 18, 2020. Available from: https://www.mon.bg/upload/24492/zap3310_maski_18112020.pdf

14 **Badillo-Goicoechea E**, Chang TH, Kim E, LaRocca S, Morris K, Deng X, Chiu S, Bradford A, Garcia A, Kern C, Cobb C, Kreuter F, Stuart EA. Global trends and predictors of face mask usage during the COVID-19 pandemic. *BMC Public Health* 2021; **21**: 2099 [PMID: 34781917 DOI: 10.1186/s12889-021-12175-9]

15 United informative portal. Accessed January 4, 2023. Available from: https://coronavirus.bg/bg/statistika

16 **Tomov**, Tchorbadjieff and Angelov, 2021, Age-specific mortality risk from Covid-19 in Bulgaria, Computer Science and Education in Computer Science, (accepted), [DOI: 10. 13140/RG.2.2.26678.01600/1] Last access on May 16, 2023. Available from: https://www.researchgate.net/publication/356420373_Epidemiology_of_COVID-19_spread_in_Bulgaria

17 **Delamater PL**, Street EJ, Leslie TF, Yang YT, Jacobsen KH. Complexity of the Basic Reproduction Number (R(0)). *Emerg Infect Dis* 2019; **25**: 1-4 [PMID: 30560777 DOI: 10.3201/eid2501.171901]

18 **Edridge AWD**, Kaczorowska J, Hoste ACR, Bakker M, Klein M, Loens K, Jebbink MF, Matser A, Kinsella CM, Rueda P, Ieven M, Goossens H, Prins M, Sastre P, Deijs M, van der Hoek L. Seasonal coronavirus protective immunity is short-lasting. *Nat Med* 2020; **26**: 1691-1693 [PMID: 32929268 DOI: 10.1038/s41591-020-1083-1]

19 Covid-19, Unified National Portal. (last accessed on November 12, 2022. Available from: http://www.coronavirus.bg

20 Report for genomic surveillance from 16.08.2021, National Centre for Infectious and Parasitic diseases, (last accessed on October 24 2022). Available from: https://www.ncipd.org/index.php?option=com_k2&view=item&id=627:ncov-sequencing-03082021&lang=bg

21 COVID-19 government response tracker. Available from: https://covidtracker.bsg.ox.ac.uk/ (last accessed on November 8 2021

22 **Kwiatkowski D**, Phillips PCB, Schmidt P, Shin Y. Testing the null hypothesis of stationarity against the alternative of a unit root: How sure are we that economic time series have a unit root? *Journal of Econometrics* 1992; **54**: 159-178 [DOI: 10.1016/0304-4076(92)90104-Y]

23 **Dickey DA and Fuller WA**. "Distribution of the Estimators for Autoregressive Time Series with a Unit Root". *Journal of the American Statistical Association* 1979; **74**: 427-431 [DOI: 10.1080/01621459.1979.10482531]

24 **Hyndman RJ**, Khandakar Y. "Automatic time series forecasting: The forecast package for R". *Journal of Statistical Software* 2008; **26** [DOI: 10.18637/jss.v027.i03]

25 **Brandenburg A**. Piecewise quadratic growth during the 2019 novel coronavirus epidemic. *Infect Dis Model* 2020; **5**: 681-690 [PMID: 32954094 DOI: 10.1016/j.idm.2020.08.014]

26 **Consiglio CR**, Cotugno N, Sardh F, Pou C, Amodio D, Rodriguez L, Tan Z, Zicari S, Ruggiero A, Pascucci GR, Santilli V, Campbell T, Bryceson Y, Eriksson D, Wang J, Marchesi A, Lakshmikanth T, Campana A, Villani A, Rossi P; CACTUS Study Team, Landegren N, Palma P, Brodin P. The Immunology of Multisystem Inflammatory Syndrome in Children with COVID-19. *Cell* 2020; **183**: 968-981.e7 [PMID: 32966765 DOI: 10.1016/j.cell.2020.09.016]

27 **Payne AB**, Gilani Z, Godfred-Cato S, Belay ED, Feldstein LR, Patel MM, Randolph AG, Newhams M, Thomas D, Magleby R, Hsu K, Burns M, Dufort E, Maxted A, Pietrowski M, Longenberger A, Bidol S, Henderson J, Sosa L, Edmundson A, Tobin-D'Angelo M, Edison L, Heidemann S, Singh AR, Giuliano JS Jr, Kleinman LC, Tarquinio KM, Walsh RF, Fitzgerald JC, Clouser KN, Gertz SJ, Carroll RW, Carroll CL, Hoots BE, Reed C, Dahlgren FS, Oster ME, Pierce TJ, Curns AT, Langley GE, Campbell AP; MIS-C Incidence Authorship Group, Balachandran N, Murray TS, Burkholder C, Brancard T, Lifshitz J, Leach D, Charpie I, Tice C, Coffin SE, Perella D, Jones K, Marohn KL, Yager PH, Fernandes ND, Flori HR, Koncicki ML, Walker KS, Di Pentima MC, Li S, Horwitz SM, Gaur S, Coffey DC, Harwayne-Gidansky I, Hymes SR, Thomas NJ, Ackerman KG, Cholette JM. Incidence of Multisystem Inflammatory Syndrome in Children Among US Persons Infected With SARS-CoV-2. *JAMA Netw Open* 2021; **4**: e2116420 [PMID: 34110391 DOI: 10.1001/jamanetworkopen.2021.16420]

28 **Michos A**, Savvidou P, Syridou G, Eleftheriou E, Iosifidis E, Grivea I, Spoulou V, Galanakis E, Syrogiannopoulos G, Tsolia M, Roilides E, Papaevangelou V. SARS-CoV-2 molecular testing in Greek hospital paediatric departments: a nationwide study. *Epidemiol Infect* 2021; **149**: e70 [PMID: 33622430 DOI: 10.1017/S0950268821000455]

29 **Nikolopoulou GB**, Maltezou HC. COVID-19 in Children: Where do we Stand? *Arch Med Res* 2022; **53**: 1-8 [PMID: 34311990 DOI: 10.1016/j.arcmed.2021.07.002]

30 **Leidman E**, Duca LM, Omura JD, Proia K, Stephens JW, Sauber-Schatz EK. COVID-19 Trends Among Persons Aged 0-24 Years - United States, March 1-December 12, 2020. *MMWR Morb Mortal Wkly Rep* 2021; **70**: 88-94 [PMID: 33476314 DOI: 10.15585/mmwr.mm7003e1]

31 Ministry of health – What parents should know for their children who go to school. Available from: https://www.mh.government.bg/bg/novini/aktualno/kakvo-tryabva-da-znayat-roditelite-na-deca-posesha/ - last accessed at 15th of January 2022

32 **Tomov LP**, Velikova TV, Batselova HM. Health risk management in kindergartens, schools and universities, during COVID-19 pandemic: a heuristic framework. *American International Journal of Biology and Life Sciences* 2021; **3**: 40-43 [DOI: 10.46545/aijbls.v3i1.207]

33 **Georgiev DS**, Batselova HM, Kotsev SV, Velikova TV. Reopening of Schools in Bulgaria during COVID-19 Pandemic: 2021 Edition. *Int J Adv Res MicroBiol Immunol* 2020; **2**: 20-21

34 **Tomov LP**, Batselova HM, Velikova TV. COVID-19 Delta Wave Caused Early Overburden of Hospital Capacity in the Bulgarian Healthcare System in 2021. *Healthcare (Basel)* 2022; **10** [PMID: 35455778 DOI: 10.3390/healthcare10040600]

35 Population by statistical regions, age, place of residence and sex. Data for demographics for 2020. Available from: https://www.nsi.bg/en/content/2977/population-statistical-regions-age-place-residence-and-sex

36 Portal for open data. Available from: https://data.egov.bg/covid-19?section=8&subsection=16&item=36

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CORRECTION

Correction to "Performance of a serological IgM and IgG qualitative test for COVID-19 diagnosis: An experimental study in Brazil"

Fabrício Freire de Melo, Lilian Martins Oliveira Diniz, José Nélio Januário, João Fernando Gonçalves Ferreira, Rafael Santos Dantas Miranda Dórea, Breno Bittencourt de Brito, Hanna Santos Marques, Fabian Fellipe Bueno Lemos, Marcel Silva Luz, Samuel Luca Rocha Pinheiro, Gifone Aguiar Rocha, Dulciene Maria de Magalhães Queiroz

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Fabrício Freire de Melo, Breno Bittencourt de Brito, Fabian Fellipe Bueno Lemos, Marcel Silva Luz, Samuel Luca Rocha Pinheiro, Instituto Multidisciplinar em Saúde, Universidade Federal da Bahia, Vitória da Conquista 45029-094, Bahia, Brazil

Lilian Martins Oliveira Diniz, Departamento de Pediatria, Universidade Federal de Minas Gerais, Belo Horizonte 30130-100, Minas Gerais, Brazil

José Nélio Januário, Departamento de Clínica Médica, Universidade Federal de Minas Gerais, Belo Horizonte 30130-100, Minas Gerais, Brazil

João Fernando Gonçalves Ferreira, Gifone Aguiar Rocha, Dulciene Maria de Magalhães Queiroz, Laboratory of Research in Bacteriology, Universidade Federal de Minas Gerais, Belo Horizonte 30130-100, Minas Gerais, Brazil

Rafael Santos Dantas Miranda Dórea, Laboratory of Bacteriology, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte 30130-100, Brazil

Hanna Santos Marques, Campus Vitória da Conquista, Universidade Estadual do Sudoeste 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 Hormindo Barros, 58 - Candeias, Vitória da Conquista 45029-094, Bahia, Brazil. freiremeloufba@gmail.com

Abstract

Correction to "Freire de Melo F, Martins Oliveira Diniz L, Nélio Januário J, Fernando Gonçalves Ferreira J, Dórea RSDM, de Brito BB, Marques HS, Lemos FFB, Silva Luz M, Rocha Pinheiro SL, de Magalhães Queiroz DM. Performance of a serological IgM and IgG qualitative test for COVID-19 diagnosis: An experimental study in Brazil. *World J Exp Med* 2022; 12(5): 100-103 [PMID: 36196438 DOI: 10.5493/wjem.v12.i5.100]". In this article, we identified an issue with the "Acknowledgments" section. Here, we then provide a recognition section for our supporting institutions.

Key Words: Correction; COVID-19 serological testing; Serologic tests; Rapid diagnostic

tests; SARS-CoV-2; Published erratum

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Core Tip: This manuscript is to provide an "Acknowledgments" section to "Freire de Melo F, Martins Oliveira Diniz L, Nélio Januário J, Fernando Gonçalves Ferreira J, Dórea RSDM, de Brito BB, Marques HS, Lemos FFB, Silva Luz M, Rocha Pinheiro SL, de Magalhães Queiroz DM. Performance of a serological IgM and IgG qualitative test for COVID-19 diagnosis: An experimental study in Brazil. *World J Exp Med* 2022; 12(5): 100-103 [PMID: 36196438 DOI: 10.5493/wjem.v12.i5.100]".

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TO THE EDITOR

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In the original publication of the article[1], we identified an issue with the "Acknowledgments" section. In that respect, we would like to acknowledge the support of Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Edital 001/2020 - Programa Emergencial de Apoio a Ações de Enfrentamento da Pandemia Causada pelo Novo Coronavírus. Furthermore, we would like to extend our thanks to PPSUS - Programa Pesquisa para o SUS - Headline 02/2020, Term of Grant nº SUS0025/2021. de Magalhães Queiroz DM and Freire de Melo F are research fellows of the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-) - Brazil. Lemos FFB is supported by the Scientific Initiation Scholarship Programme (PIBIC) of the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB). Lastly, Silva Luz M and Rocha Pinheiro SL are supported by the Scientific Initiation Scholarship Programme (PIBIC) of the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-) - Brazil. This correction will have no influence on the interpretation of the results and conclusion of this study. We apologize for any inconvenience this may cause.

FOOTNOTES

Author contributions: All authors equally contributed to this paper with conception and design of the study, literature review and analysis, manuscript drafting, critical revision, and editing, and approval of the final version.

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Country/Territory of origin: Brazil

ORCID number: Fabrício Freire de Melo 0000-0002-5680-2753; Lilian Martins Oliveira Diniz 0000-0003-1410-003X; Rafael Santos Dantas Miranda Dórea 0000-0002-5347-6913; Breno Bittencourt de Brito 0000-0002-1831-7909; Hanna Santos Marques 0000-0001-5741-1570; Fabian Fellipe Bueno Lemos 0000-0002-4686-7086; Marcel Silva Luz 0000-0003-1650-5807; Samuel Luca Rocha Pinheiro 0000-0002-8877-892X; Gifone Aguiar Rocha 0000-0002-1858-3166; Dulciene Maria de Magalhães Queiroz 0000-0003-1334-9423.

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1 **Freire de Melo F**, Martins Oliveira Diniz L, Nélio Januário J, Fernando Gonçalves Ferreira J, Dórea RSDM, de Brito BB, Marques HS, Lemos FFB, Silva Luz M, Rocha Pinheiro SL, de Magalhães Queiroz DM. Performance of a serological IgM and IgG qualitative test for COVID-19 diagnosis: An experimental study in Brazil. *World J Exp Med* 2022; **12**: 100-103 [PMID: 36196438 DOI: 10.5493/wjem.v12.i5.100]

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Pratishtha B Chaudhari, Manager Medical Operations, Amgen Asia, Amgen Asia Holding Ltd., Tokyo 1070062, Japan

Akshat Banga, Sawai Man Singh Medical College, Jaipur 302004, India

Corresponding author: Pratishtha B Chaudhari, MBBS, MD, Manager Medical Operations, Amgen Asia, Amgen Asia Holding Ltd., 4-11-8, Minami Aoyama, Minato-ku, Tokyo 1070062, Japan. pratishthabanga@gmail.com

Abstract

When conducting a literature review, medical authors typically search for relevant keywords in bibliographic databases or on search engines like Google. After selecting the most pertinent article based on the title's relevance and the abstract's content, they download or purchase the article and cite it in their manuscript. Three major elements influence whether an article will be cited in future manuscripts: the keywords, the title, and the abstract. This indicates that these elements are the "key dissemination tools" for research papers. If these three elements are not determined judiciously by authors, it may adversely affect the manuscript's retrievability, readability, and citation index, which can negatively impact both the author and the journal. In this article, we share our informed perspective on writing strategies to enhance the searchability and citation of medical articles. These strategies are adopted from the principles of search engine optimization, but they do not aim to cheat or manipulate the search engine. Instead, they adopt a reader-centric content writing methodology that targets well-researched keywords to the readers who are searching for them. Reputable journals, such as *Nature* and the *British Medical Journal*, emphasize "online search-ability" in their author guidelines. We hope that this article will encourage medical authors to approach manuscript drafting from the perspective of "looking inside-out." In other words, they should not only draft manuscripts around what they want to convey to fellow researchers but also integrate what the readers want to discover. It is a call-to-action to better understand and engage search engine algorithms, so they yield information in a desired and self-learning manner because the "Cloud" is the new stakeholder.

Key Words: Medical Subject Headings; Key words; Search engine optimization; Access; Citation; Impact factor

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Core Tip: Reputable journals like *Nature* and *British Medical Journal* lay emphasis on 'online search-ability' of articles in their author guidelines. This article urges medical colleagues to 'look inside-out' when drafting manuscript – to not only draft manuscripts around what we want to tell fellow researchers, but rather draft it in such a way that it embeds well what they are looking for. We hope that following these best practices will make it easier for search engines to crawl, index, and understand your articles to present them higher on the web-based-search results. Employing these strategies are often about making small modifications to the manuscript.

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INTRODUCTION

The famous joke, *"Where should you bury something that you do not want people to find? - On the second page of Google,"* carries an important message for medical researchers on what the authors need to pay close attention to. Studies show that nearly 75% of users never scroll past the first page of search results when searching for general information on Google[1]. Additionally, the first position on Google's organic search results has a 32% click-through rate[2].

In medical literature, when performing a literature review for draft manuscript "A", the usual sequence of actions involves searching for keywords within bibliographic databases or search engines like Google. After selecting the most relevant article "B" based on the title's relevance and reading the abstract's content, they download or purchase the article and cite it in their manuscript "A." In this process, three major elements drive the reader to ultimately cite the article "B": The keyword, the title, and the abstract. This indicates that title, abstract, and keywords are "key dissemination tools" for research papers. If these three elements are not determined judiciously by authors, it may adversely affect the manuscript's retrievability, readability, and citation index, which can negatively impact both the author and the journal.

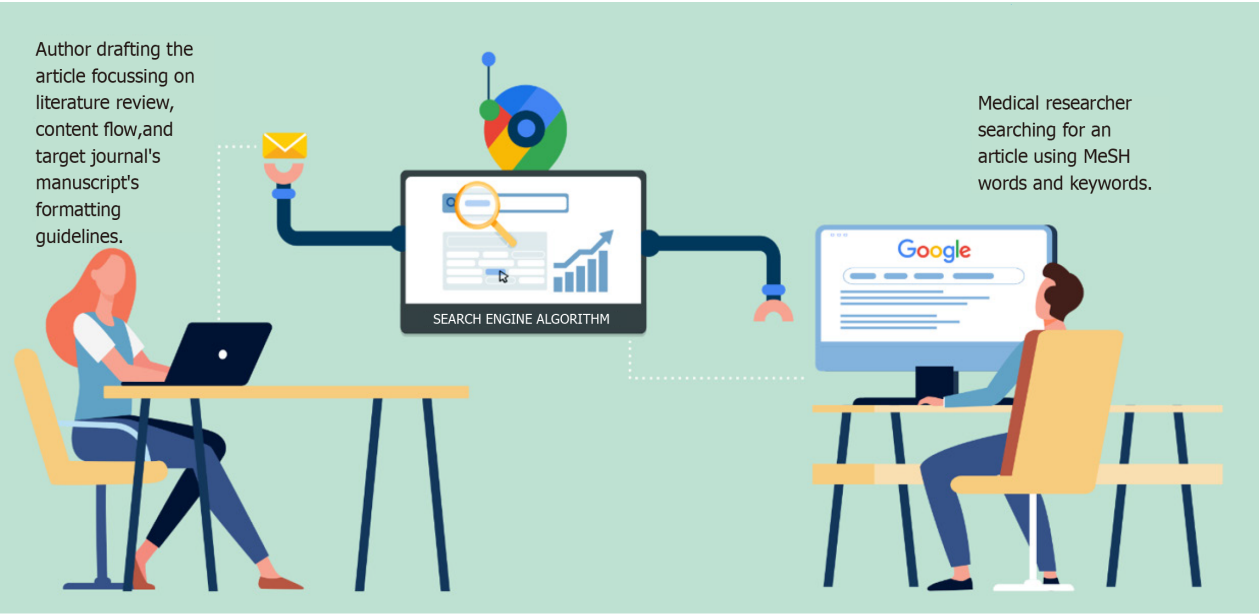
By comparison, when an author writes an article "B" that deserves to be cited in another article "A," their efforts are mainly focused on conducting a literature review, ensuring a content flow, and meeting the target journal's manuscript formatting requirements. As a result, "keywords", "title," and "abstract" are often considered the last steps in this exhaustive process and are sometimes trivialized or obliviously determined while submitting the manuscript in the journal's portal.

The success of the author is typically measured by the number of manuscripts they have authored and the number of citations those articles have received. However, for an article to be cited, it must be retrievable by readers through a search engine[3]. It is evident from the above comparison that the medical community has not yet fully explored the potential offered by search engines to rank higher for specific search terms within the vast expanse of the internet and thereby enhance access to medical literature. This discrepancy between the approach of embedding keywords when an author drafts an article versus when a reader searches for it is illustrated in Figure 1.

In this manuscript, we share our informed perspective on writing strategies that can enhance the searchability and citation of medical articles. These strategies are adapted from the principles of search engine optimization (SEO) in content marketing. We do not intend to cheat or manipulate the search engines but rather adopt a reader-centric content writing methodology that embeds well-researched keywords that are targeted towards those readers who are searching for them, thereby enhancing the article's discoverability and reach. The central theme of this article is to encourage medical authors to shift their perspective and "look inside-out" when drafting manuscripts. In other words, they should not only draft manuscripts around what they want to convey to fellow researchers but also integrate what the readers want to discover. Within this manuscript, we describe the medical article indexing systems and propose strategies for enhancing the retrievability of published articles across search engines. A tabular comparison with the non-medical content writing strategies of SEO is also included.

SETTING THE BACKDROP: WHAT ARE THE MEDICAL MANUSCRIPT INDEXING SYSTEMS?

For brevity, the indexing systems employed by PubMed and Google are discussed here.



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Figure 1 The discrepancy between the approach of embedding keywords when an author drafts an article *vs* when a reader searches for it.

PubMed

PubMed is a web-based resource that provides free access to research publications. It is an excellent first choice to review medical research topics, as it indexes over 5000 journals and nearly 30 million journal article references, covering all of the pre-clinical sciences and biomedicine. Phrases in PubMed are recognized through the subject translation table used in the system's Automatic Term Mapping. For example, if we enter "fever of unknown origin," PubMed recognizes this phrase as a Medical Subject Headings (MeSH) term. The National Library of Medicine (NLM) created a controlled, pre-defined, hierarchically organized vocabulary called the MeSH thesaurus, which is used for searching, indexing, and listing biomedical and health-related information. MeSH includes the subject headings appearing in MEDLINE, PubMed, the NLM Catalog, and other NLM databases. When standardized terms are used to search a topic, all the articles indexed in NLM's PubMed and MEDLINE are retrieved, resulting in an increase in citations for the article. Controlled vocabularies are systematic, hierarchical arrangements of words and phrases designed to describe and categorize the major subject concepts and conditions contained within a database. They can be different for different databases. The hierarchical nature of these lists narrows the search to fewer yet more specific results, keeping it consistent within that framework. Before adding an item to a database or catalog, the subject matter is determined, and specific terms that apply to those subjects are chosen from a predetermined list, regardless of the terminology used by the author within the item. The listing is standardized and predictable to an extent, ensuring a uniform system for retrieving the same concepts even when different terminology is used. For example, the term "heart attack" is always listed as "myocardial infarction" within a controlled vocabulary structure such as MeSH, the vocabulary used by MEDLINE. The disadvantages of MeSH are enumerated in Table 1.

PubMed uses a hierarchical structure to display MeSH terms, which includes broader and narrower "descriptors." The top level of the MeSH tree structure consists of 16 broad categories, which are not included in the MeSH data maintained and distributed by NLM. However, they can be used to search PubMed by using the search term "category." For example, searching for "anatomy category" will retrieve all citations indexed under any MeSH descriptor in the "A" category (anatomy). When using a MeSH descriptor to search, PubMed automatically searches for narrower descriptors listed under it in the MeSH tree structures. For example, searching "musculoskeletal neoplasm non-Hodgkins lymphoma" will show the following two trees displayed with catalog numbers called tree numbers.

Each article citation is associated with a set of MeSH terms that describe the content of the citation. When conducting a literature search, using MeSH entry terms instead of keywords can result in a more focused search and make finding more relevant citations easier. For example, if we want to search "non-Hodgkins lymphoma", finding the corresponding MeSH term can help us narrow down our results. The actual MeSH term for this topic is "non-Hodgkins lymphoma," which is further subdivided into additional categories. Our search term may fall under multiple categories, such as neoplasms and immune system diseases, as shown in the example below. Sometimes, users may begin their search with a specific MeSH term; for example, they may start with "non-Hodgkins lymphoma," but then realize

Table 1 Disadvantages of Medical Subject Headings

Disadvantages of MeSH
1 MeSH terms are manually assigned to articles after an article is made available on PubMed. Manually assigned MeSH terms are not available for recently published articles. This is a time-consuming process, and hence, these articles fail to appear in search results when only MeSH terms are used for searching
2 In a scenario where no appropriate MeSH term is found while searching for a concept, it becomes important to search for relevant words in the title and abstract as well
3 Mesh search allows us to restrict our PubMed search to only find articles where the MeSH term is the main topic. In this way, articles in which the term and any selected subheadings are indexed as a major topic are displayed. For example, searching for "mechanical ventilation"[Majr] will exclude articles where this topic is covered to a lesser extent from the search results
4 Since PubMed only contains abstracts of articles, not full texts, searching the entire article for words is not possible with the use of MeSH
5 Sometimes, PubMed might fail to automatically match the exact MeSH term with the search
6 Additionally, some readers might not be comfortable using Boolean operators and logic. Boolean operators are not recognized by all databases, and the availability of all operators in a database might also be questionable
7 Moreover, databases also differ in their way of using syntax to enter a Boolean operator. For example, in Scopus, "NOT" is entered as "AND NOT."
8 Certain databases or search systems do not affect search results based on whether the terms are entered in uppercase or lowercase, but some engines like Google Scholar may require capitalized Boolean operators for proper functioning

MeSH: Medical Subject Headings.

they really want information on "lymphoma" in general. By using the MeSH tree structure, users can easily access a complete search on any term within the hierarchical tree with a single MeSH term.

Google

Google is the world's most popular search engine, which stores all web pages in its index. The content and location (URL) of a page are described by its index entry. The process of identifying new or updated web pages is called "Crawling." Google discovers web page locations (URLs) by many different means, including tracing links and reading sitemaps, among others. As Google crawls the web, it looks for new pages and indexes them when appropriate. A crawler is automated software that crawls (fetches) pages from the web and indexes them.

Google websites are evaluated for their medical reliability using the "Health On Net" or "HON" code. HON is a non-profit organization that promotes transparent and reliable health information dissemination online and acts as a quality marker for online health information. Regular assessments are carried out by medical experts with great vigilance to provide reliable information. The HON Foundation is a non-governmental organization officially related to the World Health Organization, which carries out certifications.

Google releases "search quality rating guidelines" periodically to ensure that search engines provide a diverse set of reliable, high-quality search results, presented in the most helpful order. Medical content falls under the category of "Your Money or Your Life" pages. This category includes all information that could potentially impact the future happiness, health, financial stability, or safety of the individual. Google is responsible for representing such information in the required indexing manner and has very high rating standards for such pages. When Google search quality raters evaluate a website, they are guided to look for expertise, authority, and trustworthiness, abbreviated as "EAT". Their ratings are further augmented by the algorithm, and that is when the information is displayed in a responsible manner.

WRITING STRATEGIES TO AUGMENT THE SEARCHABILITY OF PUBLISHED MANUSCRIPTS

Here are some strategies to use when creating the manuscript with the intent of ranking well in the search engines and reaching the relevant readers, ultimately yielding a higher number of clicks, downloads, and citations:

MeSH keyword gap analysis during the manuscript drafting phase

To increase the chances of an article being found and cited, we suggest conducting a "MeSH keyword gap analysis" at the beginning of the manuscript writing process. This approach can potentially improve the retrievability and citation of the article. Currently, most authors only determine and mention keywords during the manuscript submission stage without putting detailed thought into it,

which may result in missing out on strategic keywords.

Awareness of the journals and their role in keyword optimization

The wider reach of an article is not only important for the individual author but also beneficial for the journals. It helps the journals boost their impact factor, which is a scientometric measure of the frequency with which the average article in that journal has been cited in a particular year. The impact factor is used as a proxy for the importance or rank of a journal by calculating the number of times its articles are cited. Therefore, higher citation rates are beneficial for the ranking and credibility of journals, which implies they must focus on keyword optimization as well. The journals should hire professionals with an SEO background who are able to analyze the manuscripts for optimal use of keywords.

The list below complies the author's instructions for keywords for selected journals. Except for *Nature* and the *British Medical Journal* (BMJ), none of the journals mention the importance of searchability, page ranking, or SEO in their author guidelines. It is evident that currently there is not much emphasis on the optimized use of keywords, their selection, or placement. The only exception is *Nature* publishing house, whose journal author guidelines state that "*We ask authors to be aware of abstracting and indexing services when devising a title for the paper: providing one or two essential keywords within a title will be beneficial for web-search results*". They also suggest authors "*choose keywords to maximize visibility in online searches as well as being suitable for indexing services*"[4]. Meanwhile, the *BMJ* authors hub highlights that "*it is essential for your paper to be correctly set up for discoverability, right from the start*", and provides six steps to make the work more visible and, as a result, more likely to be cited.

Author instructions for keywords within a few sampled high-impact factor journals

New England Journal of Medicine[5]: Three to 10 keywords or short phrases should be added to the bottom of the abstract page, which will assist us in indexing the article and which may be published with the abstract. Use terms from the MeSH in *Index Medicus* when possible.

PLOS One[6]: Add keywords to help expedite the processing of your manuscript (optional). You will not have an opportunity to make changes, so make sure to add concise, accurate keywords now.

Journal of Clinical Oncology[7]: Immediately after the abstract, provide a maximum of six keywords, using British spelling and avoiding general and plural terms and multiple concepts (avoid, for example, "and" and "of"). Be sparing with abbreviations; only those firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Annals of Internal Medicine[8]: No mention or instructions on keywords.

BMJ[9]: "*It is essential for your paper to be correctly set up for discoverability, right from the start*", and it provides some steps to make the work more visible and, as a result, more likely to be cited.

Keywords selection

Keywords play a crucial role in determining the discoverability of an article, leading to a chain reaction that includes clicking through, dwelling on, downloading, and ultimately citing the article. Citation is the critical element that generates the greatest impact factor. Keywords help readers find the best-suited article aligned with their research question and enhance the discoverability of research articles by ensuring that papers are substantially indexed by databases and search engines. It is important to select well-chosen keywords that best represent the article content and include various interchangeable terms and synonyms, be they in abbreviated or phrase form, making it easier for the manuscript to be easily identified and cited. The keywords of a manuscript should be selected by authors in such a manner that, if fed into search engines, accurate articles or the book's contents are retrieved. However, keyword selection can be subjective and differ amongst authors, leading to difficulty retrieving similar articles by authors who are searching the relevant literature for citations in their future articles. If keywords are used incorrectly, it may affect the citation index of the article. Therefore, it is a good practice to do a dipstick test by searching for some keywords in PubMed and analyzing the search results that reflect similar papers as ours. It is also essential to integrate the keywords well in the title and abstract, as they may not yield the article in the results if the manuscript is paid and the full text is not visible, as the search is based on the title and abstract.

Keywords are essential components of a manuscript and are typically listed after the abstract. Most journals require authors to provide three to five keywords, although some may ask for up to eight. Therefore, it is recommended to have eight to ten relevant keywords prepared before submitting the manuscript. Unfortunately, keyword selection is often left as the last step during manuscript submission, which can lead to an inadequate selection.

The problem with keywords is that they are not standardized because authors generate them, which cannot be exact words as in contents and can vary from author to author. Thus, they may not retrieve similar articles from different authors who are searching for the same relevant literature. Conducting effective MeSH term research and drafting the manuscript based on MeSH terms can increase online

Table 2 Search engine optimization strategies and corresponding adapted medical writing strategies

S. No.	SEO strategies	Corresponding medical writing strategies
1	Backlinks to share link equity	1 Citations of articles that enhance the credibility of articles 2 Blog about the article on forums like WordPress and link to it 3 Diversified media coverage, including social media articles linking the article, podcasts, and videos 4 Use opportunities to target different web areas like YouTube, podcast portals, social media, blogging portals, news, *etc.*
2	Organizing information into categories, pages, and subpages on a website and linking them to each other is called site architecture or information architecture	Have impactful headings using keywords within the headings
3	The flatter the site's structure is (requiring a lesser number of clicks to reach the desired information), the better its opportunity to rank well	MeSH terms and keywords search a manuscript by searching its abstract. Also, the abstract is commonly the only publicly available resource of an article, making it easily searchable for those on Google. Hence, incorporate critical keywords in the abstract of the article
4	The title tag is the most important on-page element to optimize for keywords, so the post has the best chance of ranking for a given keyword if that keyword appears at the beginning of the title tag (often the post headline). Also, make sure other posts linking to this one use this keyword as their link text (what SEOs call "anchor text")	Incorporate keywords in the title of the article. Give it some thought and brainstorm with the writing team about what keywords a potential researcher will search for. Use alternative terms and broader terms throughout the text to describe the concepts the authors are writing about because too specific terms may not filter in the search method
5	An average user looks at two things before opening a webpage: headlines and meta descriptions. The latter is always displayed below the webpage title in Google search results. Search engines highlight keywords in the meta description, thus making it a genuine call-to-action booster—it encourages people to click the link and check out the content	Meta description corresponds to the abstract of the article, particularly the objective and the initial three to four sentences
6	Carefully-designed keyword strategies to make more impact on Google's search engine algorithms	Research and integrate MeSH terms and keywords
7	Any time someone visits the website, we want them to have a positive user experience and engage with the site content	Lucid story-telling and point-to-point writing methodology
8	Visuals: when setting up pages, it is important to ensure that the pictures are always readable. "Alt" tags provide valuable details for the target audience. Google cannot understand images solely as image files	Explain the paper and integrate good-quality images and tables. Develop "at-a-glance" infographics covering key messages on the manuscript in a snapshot. Utilize digital designers, draw on easy digital tools like procreate, or hand draw and scan high-resolution images and convert them digitally to digitized images. Purchase editable vector images and templates. Determine figure legends judiciously, incorporating the keywords and MeSH terms, so their searchability is enhanced on Google Images
9	Internal linking is one of the SEO fundamentals that influence the dwell time of the target audience on the page	Generate the interest of the readers and direct them to various sections of the article by numbering the sections and structuring the topic well to enhance the time they spend on the page and article

MeSH: Medical Subject Headings; SEO: Search engine optimization.

traffic to the articles from the medical community more efficiently.

Research and use related search terms

Using synonyms and variations (related terms) of the keywords can help improve the discoverability of an article in the search results. For example, a reader looking for information on "heart attacks" may use search terms such as "heart attacks," "myocardial infarction," "myocardial infarctions," and so on. Therefore, when writing an article, it is important to embed various search terms that a researcher might use when searching for the relevant information that the article provides. Furthermore, it is important to pay attention to the terminologies that prevail in different geographies and how certain concepts and conditions are referred to based on medical, academic, and cultural backgrounds in that region. This information can be discovered through social listening and integrated into the manuscript. Although not yet widely practiced or emphasized, it is important to be mindful of MeSH keyword integration before and during manuscript writing. MeSH keywords may be searched, and all relevant words in the same hierarchy and those above and below it can be retrieved. It will help to identify keywords in the MeSH thesaurus that can be integrated throughout the article or abstract and title to perform well in the search engine algorithm.

Title and abstract

The ability to accurately highlight the core content of an article is crucial for crafting an impactful research paper title. A good title typically consists of a 10- to 12-word phrase with descriptive terms. Indexing agencies use the words used in the title to tag the article and make it easily discoverable. Making the title and abstract more action-oriented, concise, and less wordy can improve the search quality. Moreover, keywords provide an opportunity to tag the article with more relevant terms. Therefore, it is not a wise choice to use similar words or phrases both in the title and keywords.

The method of conducting a literature search differs from one person to another, which may result in different articles or a different number of articles despite looking for similar topics of interest. It is not always possible to predict how others will search the same literature. However, it is possible to elucidate how authors themselves might search the same literature. Authors should attempt to apply search strategies similar to those adopted by a reader or peer. Authors can utilize Google Trends to gain knowledge of internet search trends and discover the latest search trends for a particular word or phrase. Google Trends also helps in comparing search trends between different words and phrases, which helps authors choose the most relevant one for their title and keywords. From the perspective of the audience, study what types of topics the target audience would search for and that we would want our article to be found for and cited for.

Comparatively, an abstract is meant to provide a brief and accurate summary of the study, to aid the judgment of the reader of whether they should read the rest of the paper or not. A good understanding of the intent of the readers is foundational to this.

Effective visuals

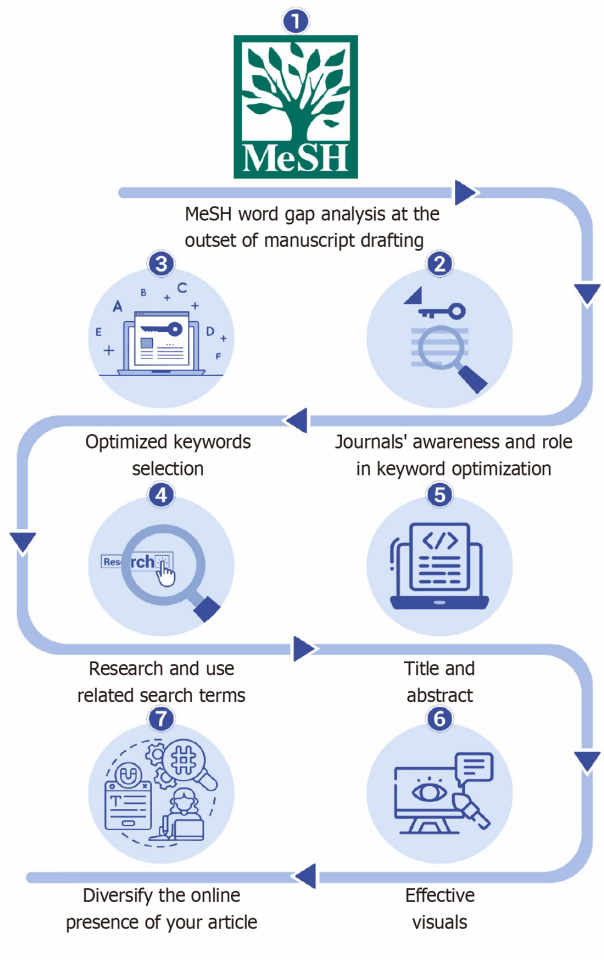
Effective visuals help with readability and storytelling, greatly supporting learning, adding value, and keeping the reader engaged with the article. Vivid imagery is a much more effective and efficient method of communication than plain text. Articles that include images appear at the forefront of search engine results and have a viewership of up to 94% more often than those without images. Article visuals include tables, figures, clinical images, infographics, *etc*. Such elements can draw visual learners to our article. We can aid in the discovery process by making sure that our images and our site are optimized for Google Images. People who do not want to read an entire article but are still interested in the material can glance at these images and absorb valuable information without losing time. This helps engage and attract citations from readers who might have otherwise passed them by.

Images are proven to attract significantly more views compared to those without images, with a staggering 94% increase in views. Moreover, up to 60% of the population are visual learners, which means they prefer visual content over textual content. This is because the human brain is wired to process images faster than plain text, with images being processed 60000 times faster than text. Visuals are often available freely, even if an article is behind a paywall, and may even feature on Google Images, bringing in organic traffic to the article. It is important to carefully determine the figure legend and title by integrating the key MeSH terms. The average attention span is only around eight seconds, and visual content allows for valuable information to be distributed in a format that can be easily understood within this timeframe. In fact, according to the Massachusetts Institute of Technology, the human brain can process images in as little as 13 milliseconds. Good-quality visuals, developed with the support of professionals such as graphic designers, can lead to higher-quality traffic to the website by conveying valuable information in an engaging visual format. However, it is important to ensure that the visuals are original, as dealing with a copyright lawsuit can be an expensive affair.

Diversify the online visibility of the article

In addition to being an educational article intended for an academic and research audience, our manuscript will also be published as a webpage. A significant proportion of researchers and academicians search for articles not only on PubMed or MEDLINE but also on Google. Google attracts such traffic not only by analyzing words typed into its search box in a browser but also by using the words spoken to a mobile phone or assistant device and through "search engine auto-complete" features. This furthermore emphasizes the importance of the author's mindful efforts towards 'keywords and Google's searchability of them when drafting the manuscript.

To increase the article's online visibility, it is recommended to promote it through various channels, such as blogging, patient education, hospitals, and social media, along with linking the article. With educational content delivered in a conversational tone, our prospects get to know our target readers in a more personal way. Blogging is a particularly effective way to engage with readers, as more and more people are turning to blogs for information and sharing interesting articles on their social media networks. To leverage this behavior, it is crucial to cultivate the habit of blogging and maintain an active presence on all the relevant social media networks. Furthermore, some portals allow the authors to showcase unpublished, non-peer-reviewed articles that are ahead of print for the purpose of early clinical adoption and information sharing. However, it is only available for a limited time, mostly until publication. The SEO strategies and corresponding adapted medical writing strategies are enumerated in Table 2. Figure 2 showcases the proposed strategies for enhancing the searchability of manuscripts.



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Figure 2 Proposed strategies for enhancing the searchability of manuscripts—The art of being cited. MeSH: Medical Subject Headings.

LIMITATIONS

The discussion applies exclusively to the online readership, and we have naturally excluded from consideration those readers who read articles in journals in a hard copy format. The study assumed that clinicians would research information on their hand-held mobile devices and laptops in greater numbers, more so after the onset of the coronavirus disease-2019 pandemic. Similarly, information stored in textbooks is not included in this discussion. To some extent, Google Books information is included.

CONCLUSION

The medical community primarily searches for articles on PubMed using keywords and MeSH terms. However, not many authors write their articles with searchability in mind. Ideally, an article that answers a specific research question with a large sample size and rigorous research methodology should appear on the first page of the search engine. In reality, it is dependent on the accurate use of keywords, and one has to sift through dozens of articles to find the most well-matched article.

To enhance the visibility of an article, medical authors and publishers use various strategies, such as "open access availability of articles" and tagging articles in blogs and multiple media formats, including "podcasts, audio, and video formats." In addition to these existing practices, authors can improve the searchability of their articles by educating themselves about "Google keywords" and MeSH search and strategically embedding those words throughout the article. The point of this article is not to promote articles irrationally but to emphasize the importance of understanding the principles of search engine algorithms to enhance organic readership and uptake of the article.

We hope that following these best practices will make it easier for search engines to crawl, index, and understand the articles to rank them higher in the search results. Employing these strategies often involves making small modifications to the manuscript. When observed individually, these changes

might seem like incremental improvements, but when combined with other optimizations, they could have a noticeable impact on the article's performance in organic search results. The time has come that we understand and engage the search engine algorithms better, so they yield information in a desired and self-learning manner.

We acknowledge that the consumption of medical updates by healthcare professionals has become more digital in the past few years. Due to this, the updates we receive are also determined by a new 'factor' - the internet and its search engine which yields information based on the search words it is fed. Hence when drafting a manuscript this new factor must be considered along with technical accuracy of drafting research and grammatical flow. Hence not only the journal and the readers but also the "Internet" or the "cloud" is also a stakeholder in this process.

FOOTNOTES

Author contributions: Chaudhari PB and Banga A contributed equally to this work; Chaudhari PB and Banga A conceptualized the manuscript theme and outline; Chaudhari PB and Banga A performed the literature search and wrote the manuscript; All authors have read and approve the final manuscript.

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Country/Territory of origin: Japan

ORCID number: Pratishtha B Chaudhari 0000-0002-8060-217X; Akshat Banga 0000-0003-2626-8219.

S-Editor: Liu JH
L-Editor: A
P-Editor: Ju JL

REFERENCES

1 **Kagan M**. 100 Awesome Marketing Stats, Charts, & Graphs. HubSpot. October 20, 2016 Available from: https://blog.hubspot.com/blog/tabid/6307/bid/14416/100-awesome-marketing-stats-charts-graphs-data.aspx
2 **Dean B**. We analyzed 4 million google search results. Here's what we learnt about organic click-through rate. BACKLINKO. October 14, 2022] Available from: https://backlinko.com/google-ctr-stats
3 **Mondal H**, Mondal S1, Mondal S2. How to Choose Title and Keywords for Manuscript According to Medical Subject Headings. *Indian Journal of Vascular and Endovascular Surgery* 2018; **5**: p 141-144 [DOI: 10.4103/ijves.ijves_15_18]
4 **Nature Portfolio**. How to publish your paper. Nature. Available from: https://www.nature.com/nature-portfolio/for-authors/publish
5 Information for Authors. *N Engl J Med* 1992; **326**: 76
6 **PLOS One**. Your step-by-step guide to the submission form. Available from: https://journals.plos.org/plosone/s/submit-now
7 **Clinical Oncology**. Author Information Pack. April 9, 2023. Available from: https://www.elsevier.com/wps/find/journaldescription.cws_home/623018?generatepdf=true
8 **Annals of Internal Medicine**. February 22, 2023. Available from: www.acpjournals.org/pb-assets/pdf/AnnalsAuthorInfo-1656457118343.pdf
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Peer Reviewer of *World Journal of Experimental Medicine*, A. Vijaya Anand, PhD, Professor, Department of Human Genetics and Molecular Biology, Bharathiar University, Coimbatore 641046, Tamil Nadu, India.
avahgmb@buc.edu.in

AIMS AND SCOPE

The primary aim of the *World Journal of Experimental Medicine* (*WJEM*, *World J Exp Med*) is to provide scholars and readers from various fields of experimental medicine with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

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INDEXING/ABSTRACTING

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REVIEW

Neurologic orphan diseases: Emerging innovations and role for genetic treatments

Ivelina P Kioutchoukova, Devon T Foster, Rajvi N Thakkar, Marco A Foreman, Brandon J Burgess, Rebecca M Toms, Eduardo E Molina Valero, Brandon Lucke-Wold

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Ivelina P Kioutchoukova, Rajvi N Thakkar, Marco A Foreman, Brandon J Burgess, Rebecca M Toms, Eduardo E Molina Valero, College of Medicine, University of Florida, Gainesville, FL 32611, United States

Devon T Foster, Florida International University Herbert Wertheim College of Medicine, Florida International University Herbert Wertheim College of Medicine, Miami, FL 33199, United States

Brandon Lucke-Wold, Department of Neurosurgery, University of Florida, Gainesville, FL 32611, United States

Corresponding author: Brandon Lucke-Wold, MD, PhD, Neurosurgeon, Department of Neurosurgery, University of Florida, 1505 SW Archer Rd, Gainesville, FL 32611, United States. brandon.lucke-wold@neurosurgery.ufl.edu

Abstract

Orphan diseases are rare diseases that affect less than 200000 individuals within the United States. Most orphan diseases are of neurologic and genetic origin. With the current advances in technology, more funding has been devoted to developing therapeutic agents for patients with these conditions. In our review, we highlight emerging options for patients with neurologic orphan diseases, specifically including diseases resulting in muscular deterioration, epilepsy, seizures, neurodegenerative movement disorders, inhibited cognitive development, neuron deterioration, and tumors. After extensive literature review, gene therapy offers a promising route for the treatment of neurologic orphan diseases. The use of clustered regularly interspaced palindromic repeats/Cas9 has demonstrated positive results in experiments investigating its role in several diseases. Additionally, the use of adeno-associated viral vectors has shown improvement in survival, motor function, and developmental milestones, while also demonstrating reversal of sensory ataxia and cardiomyopathy in Friedreich ataxia patients. Antisense oligonucleotides have also been used in some neurologic orphan diseases with positive outcomes. Mammalian target of rapamycin inhibitors are currently being investigated and have reduced abnormal cell growth, proliferation, and angiogenesis. Emerging innovations and the role of genetic treatments open a new window of opportunity for the treatment of neurologic orphan diseases.

Key Words: Neurologic orphan diseases; Gene therapy; Clustered regularly interspaced palindromic repeats/Cas9; Antisense oligonucleotides; Adeno-associated virus; mTOR inhibitors

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Core Tip: Neurologic orphan diseases are rare conditions that impact a small percentage of the population. Through new advances in technology and research, the use of genetic treatment for these conditions is increasing. Recent advances in clustered regularly interspaced palindromic repeats/Cas9, adeno-associated viral vectors, antisense oligonucleotides, and mammalian target of rapamycin inhibitors have shown improvements in the care of patients and their quality of life.

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INTRODUCTION

Orphan diseases are rare diseases that affect less than 200000 individuals within the United States[1]. Despite being rare, these diseases affect over 300 million individuals globally[2]. Of the 7000 diseases listed on the National Institute of Health's Office of Rare Diseases site, most are neurological and of genetic origin[3]. 90% of orphan diseases have serious neurological effects[2]. Neurologic orphan diseases are fatal, drastically decrease quality of life, and are defined by long periods of disability[4]. However, diagnosis and treatment of rare central nervous system (CNS) disorders pose a challenge[2]. Lack of access to diagnostic genomic sequencing, screening tests, and specialists contributes to the difficulty of diagnosing and managing neurologic orphan diseases[2]. Most neurologic orphan diseases don't have treatments that prevent disease progression[4]. Additionally, clinical trials investigating neurologic orphan disease therapeutics have the lowest success rate[5]. Global Genes, a rare disease advocacy organization, states that since 2021, a total of $22.9 billion has been invested in research on neurologic orphan diseases, which offers a promising future for patients with these conditions[6].

One study found that the use of whole genome sequencing within clinical practice increases the diagnosis of neurologic orphan diseases[7]. Genetic conditions such as Huntington's disease and Friedreich's ataxia are caused by repeat expansions which can effectively be detected by whole genome sequencing due to its high sensitivity and specificity for repeat expansions[7].

The Patient Identification and Engagement for Rare CNS Disorders initiative is designed to investigate and improve barriers to diagnosis and clinical research trials[2]. This initiative plans to address the underrepresentation of certain groups from clinical trials and to improve access for those individuals in the participation of trials investigating new gene therapy approaches[2]. The future for the diagnosis and management of neurologic orphan diseases looks promising and hopes to improve the efficacy of therapeutic agents by gene targeting methods. Some forms of gene therapy include the use of clustered regularly interspaced palindromic repeats (CRISPR)/Cas9, antisense oligonucleotides (ASO), adeno-associated viruses (AAV), and mammalian target of rapamycin (mTOR) inhibitors.

CRISPR/Cas9 is a form of gene-editing where a guide RNA binds to a target sequence of genomic DNA, followed by the endonuclease, Cas9, binding to the guide RNA. Cas9 then creates a double strand break in the genomic DNA which is then repaired. This process allows for the inclusion or exclusion of desired genes to create a desired mutation[8].

ASO are small molecules that can modify gene expression preventing or altering protein production. If a certain protein is undesired, an ASO can be designed to cause the protein to be terminated or partially expressed and modify it so that it is not harmful[9].

AAV are used as a vector for gene therapy by using a non-enveloped virus engineered to deliver deoxyribonucleic acid (DNA) to targeted cells[10]. This gene therapy has shown preclinical and clinical access in gene replacement, gene silencing, and gene editing[11].

mTOR is a kinase closely correlated with the occurrence of neurodegenerative diseases and tumors in humans. The goal of mTOR inhibition therapies is to block the mTOR signaling pathway that may be contributing to abnormal signal transduction to block the occurrence and development of disease[12].

MUSCULAR DETERIORATION

Spinal muscular atrophy

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease caused by deletions or mutations in the survival motor neuron (*SMN1*) gene[13]. Specifically, diagnostic testing commonly demonstrates the absence of SMN1 exon 7 on chromosome 5[14,15]. SMA is characterized by progressive muscle weakness and atrophy resulting from

progressive degeneration and irreversible loss of anterior horn cells in the spinal cord[16]. The severity of SMA can range from more mild cases where the onset occurs in adulthood and progresses at a slow rate to more severe cases where the onset can occur in the first months of life and result in respiratory failure[17].

In recent years, new treatment options like gene therapy involving splicing modulation of SMN2 and SMN1 genes and the development of the first approved drugs for SMA treatment have shown promise in treating SMA. When therapy is initiated early, it can significantly alter the natural course of the disease. Current evidence in these treatments is limited to a small scope of patients and more research is needed for conclusive results[17].

Nusinersen was the first drug that received approval for the treatment of SMA. Nusinersen enhances the inclusion of exon 7 in mRNA transcripts of SMN2 by suppressing the binding of certain splicing factors which results in an increase in functional SMN2-mRNA with included exon 7[18-20]. Various studies in infants and young children have displayed improvements in prolonged time of death and improved motor functions[21-23]. Furthering the potential of SMN2 gene alteration, Risdiplam, and Branaplam are oral medications that have been shown to cross the blood-brain barrier and increase the number of full-length SMN proteins[24].

Gene therapy has also shown promise with SMN1-gene replacement. Studies in mice have shown prolonged survival following successful vector delivery of intact SMN1-gene across the blood-brain barrier[25,26]. Zolgensma is a gene therapy medicine that is administered as an intravenous infusion and uses adeno-associated virus vectors to deliver a functional copy of SMN to motor neuron cells[27]. Clinical trials in children treated with Zolgensma have shown improved survival, motor function, and developmental milestones following treatment[28,29]. A summary of emerging treatment options for SMA can be found in Figure 1 below.

Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is the most common hereditary neuromuscular disease and is one of the most severe forms of inherited muscular dystrophy. Symptoms of DMD include severe and progressive muscle wasting, muscle weakness, and difficulty with movement[30]. Late stages of DMD often require the need for assisted ventilation and the use of a wheelchair to perform daily activities and lead to premature death in the mid-twenties due to respiratory muscle weakness or cardiomyopathy[31].

DMD is an X-linked inherited disorder that predominantly affects males. The onset of DMD occurs due to mutations in the dystrophin gene on chromosome Xp21. This results in a ceased production of dystrophin in cardiac and skeletal muscle. The absence of dystrophin results in a loss of myofibril membrane integrity through cycles of necrosis and regeneration. Fibrous connective tissue and fat then replace muscle over time, resulting in the progression of expressed clinical symptoms[30,32].

In recent years, there has been progress in the development of diagnosis and therapeutics for DMD, but the current treatments given do not cure DMD[33]. Daily prednisone treatment is commonly used to increase muscle strength and function, improve pulmonary function, and significantly slow the progression of weakness[34,35]. While this treatment does not cure the disease, it does improve the overall quality of life for patients.

The most advanced therapy is antisense oligonucleotides -mediated exon skipping which has shown promise in clinical application. For this technique, the administration of 20-30 bp long antisense oligonucleotide hybridizes to splice motifs necessary for pre-mRNA processing and mask RNA splicing signals. This leads to the exclusion of the intron and adjacent exon, creating an in-frame mRNA without the targeted exon. This mRNA can then be translated into a truncated and partially functional dystrophin protein[33,36].

CRISPR/Cas technology has been used therapeutically to treat DMD by upregulating a dystrophin homolog, utrophin, to compensate for the lack of dystrophin protein which has been successfully demonstrated in patient cells. In these patients, full-length dystrophin was restored in patient cells carrying duplication mutations[37]. Manipulations resulting from CRISPR-Cas9 were shown to restore the expression of truncated but partially functional dystrophin, improve skeletal and cardiac muscle function, and increase the survival of mdx mice significantly[38].

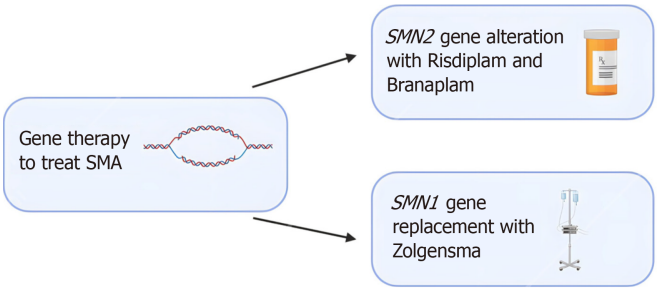
Emerging vector-mediated gene therapy in mice has been shown to deliver a functional DMD gene to cells lacking dystrophin protein and has been shown to increase exercise capacity[39]. While promising, this technique is challenging due to the very large size of the dystrophin gene and the widespread distribution of muscles[40].

EPILEPSY & SEIZURES

Dravet syndrome

Dravet syndrome (DS), first described by Charlotte Dravet in 1978, is an early-onset epileptic syndrome characterized by a variety of refractory seizures and neurodevelopmental impairment that often persist into adulthood[41,42]. The clinical features of DS typically progress over time, most commonly first presenting as a bilateral tonic-clonic in the first year of life, with half of the patients being febrile[43]. The disease progresses to multiple types of seizures, often leading to poor therapeutic control and the majority of patients (93%) experiencing status epilepticus[43,44]. Additionally, patients with DS will develop neurodevelopmental delay, as well as motor and cognitive impairment that will persist into adulthood. It should be noted, however, the diagnosis is highly clinical as both magnetic resonance imaging (MRI) and electroencephalogram (EEG) studies may be nonspecific[45].

Previously named severe myoclonic epilepsy of infancy, the molecular basis of DS arises from a *de novo* mutation on chromosome 2q24 on the sodium voltage-gated channel alpha subunit (SCN1A) gene[45,46]. Although the role of the SCN1A variant in the pathogenesis of DS has not been fully elucidated, some studies have suggested that the diffuse neuronal hyperactivity in DS patients is correlated with a loss of inhibitory GABAergic interneurons which have an



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Figure 1 Emerging gene editing treatment options for spinal muscular atrophy. Risdiplam and Branaplam are oral medications which can cross the blood-brain barrier and increase the number of spinal muscular atrophy (SMA) full length proteins by targeting the *SMN2* gene. For patients that require replacement of the *SMA1* gene, Zolgensma is an intravenous medication that uses an adeno-associated viral vector to deliver a functional copy of the gene. Clinical trials in patients treated with Zolgensma have shown positive outcomes. SMA: Spinal muscular atrophy.

SCN1A mutant in non-coding regions[47,48].

Currently, DS is managed symptomatically with a series of anti-seizure medications (valproic acid being the first line of treatment), though this regimen has variable efficacy[48]. Gene-specific therapies for the causes of DS continue to gradually emerge. One study used Targeted Augmentation of Nuclear Gene Output technology, utilizing ASO, to successfully increase the expression of the SCN1A protein in mice models. In addition to a higher expression of this SCN1A product, the incidence of seizures in these DS mice was significantly reduced[49]. The use of viral vectors to target genes has shown success in some diseases but poses a challenge in DS[50]. The SCN1A coding sequence is 6kb long, which exceeds the carrying capacity of adeno-associated viruses[50]. However, the use of a different viral vector, such as lentiviruses, poses another challenge as it demonstrates a limited spread in neural tissue therefore it cannot effectively treat large brain areas[50]. Another approach to upregulating the expression of SCN1A is through the use of dCas9-based gene activation systems. One study found that the use of dCas9 resulted in the upregulation of SCN1A in brain tissue and cultured neurons[51]. Overall, there have been significant developments in viral vectors and molecular techniques that demonstrate promising results.

Ohtahara syndrome

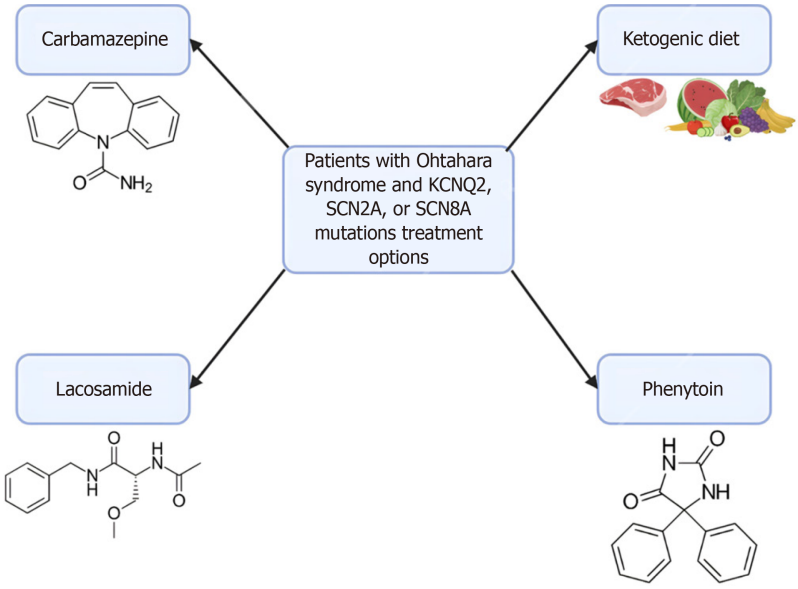
Ohtahara syndrome, also referred to as Early Infantile Developmental and Epileptic Encephalopathy (EIDEE) syndrome, is a group of devastating pediatric conditions characterized by frequent spasms in neonates and infants leading to severe cognitive and physical disabilities and even death[52,53]. There are numerous causes of Ohtahara syndrome, including structural brain defects, metabolic derangements, and genetic variants. Multiple studies have identified the most common variants associated with Ohtahara syndrome are PRRT2, SCN1A, KCNQ2, and SLC2A1[54]. The extensive distribution of these channelopathies and their various penetrance help explain the evolution of focal seizures to status epilepticus seen in EIDEE[54,55].

Seizure episodes in Ohtahara syndrome are initially treated with anti-seizure medications but are usually only in managing the frequency of seizures. Patients with Ohtahara syndrome with KCNQ2, SCN2A, or SCN8A mutations have been found to respond to sodium channel anti-seizure medications, such as carbamazepine, lacosamide, or phenytoin[56,57]. Additionally, implementing a ketogenic diet has been shown to provide some improvement in many infants[58]. Currently, the rise in genetic testing has allowed identifying the monogenic etiology of various EIDEE. An AAV-based gene replacement therapy has been proposed for Ohtahara syndrome particularly for the transmembrane sodium channel SLD13A5, though these principles could provide insight into future therapeutic targets of Ohtahara syndrome[59]. However, challenges exist for gene therapy due to the limited knowledge of the disease mechanism and progression of Ohtahara syndrome. Further research is needed in patients with Ohtahara syndrome to evaluate the efficacy of gene therapies such as viral vectors. A summary can be found in Figure 2 below.

Lennox-gastaut syndrome

Lennox-gastaut syndrome (LGS) is another severe pediatric epileptic and encephalopathic disorder characterized by severe pediatric seizures, treatment-resistant epilepsy, and cognitive impairments[60,61]. As in various other childhood epilepsy disorders, LGS is caused by several etiologies, including genetic predispositions, anatomical brain abnormalities, hypoxic-reperfusion encephalopathies, meningitis, and head trauma[61]. The majority of children affected by LGS have underlying genetic disorders, often chromosomal syndromes, or *de novo* pathogenic variants[61,62].

As LGS is characterized by seizures resistant to pharmacologic therapy, management often includes a multidisciplinary approach, often including dietary and surgical interventions. In one case series, 50% of children received a greater than 50% reduction in seizure frequency, and almost a quarter of the children achieved a greater than 90% reduction[63]. Additionally, the serotonergic agent fenfluramine is commonly used in LGS and has been shown to have significant benefits for the reduction of generalized tonic-clonic seizures and drop seizures[64]. Fenfluramine increases the level of serotonin in the extracellular compartment and acts as a serotonergic 5-HT2 receptor agonist and an alpha-1 receptor antagonist to decrease anti-epileptic activity. Since LGS seizures evolve over a patient's life, an LGS algorithm detailing several anti-epileptics medications as well as adjunctive therapy including a ketogenic diet, possible surgical resection,



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Figure 2 Treatment options for patients with Ohtahara syndrome. In patients with Ohtahara Syndrome with KCNQ2, SCN2A, or SCN8A mutations, there are several treatment options available. Carbamazepine, lacosamide, and phenytoin are sodium channel anti-seizure medications. These medications don't offer a cure and are currently only used to manage the frequency of seizures. Additionally, implementation of a ketogenic diet has been shown to reduce seizure frequency in infants

and close EEG monitoring[65].

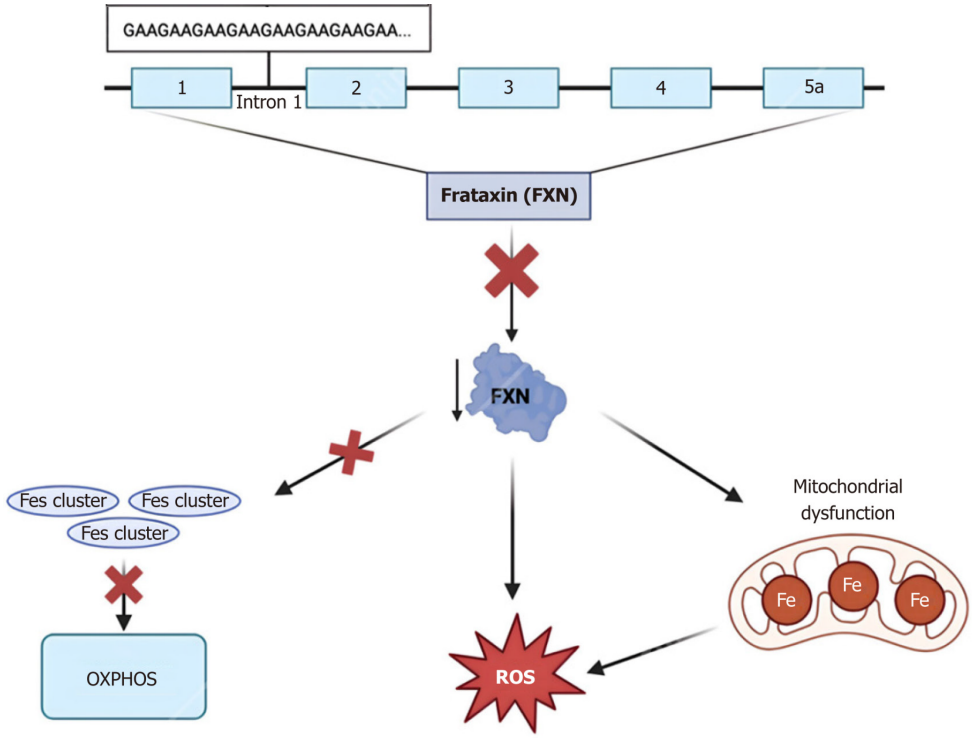
NEURODEGENERATIVE MOVEMENT DISORDER

Friedreich ataxia

In the discourse of neurologic orphan diseases, one important consideration in the context of neurodegenerative movement disorders is Friedreich Ataxia (FRDA). In the second half of the 19th century, its original description by German professor of medicine at Heidelberg, Nikolaus Friedreich, remarked FRDA was a degenerative atrophy of the posterior spinal cord columns[66]. Further, between the years of 1863-1877, Friedreich published the earliest and most extensive works on "Friedreich's ataxia", which provided insightful descriptions of this neurological disorder as being marked with the principal abnormality of axonal thinning without axonal loss of the dorsal spinal roots[66]. Unfortunately, Friedreich received little recognition for his academic effort during his lifetime, however, an anonymous obituary of "perhaps his most important work" details Friedreich's rapid scientific progress and 59 publications[67]. Nonetheless, the full extent of FRDA's etiology couldn't be fully appreciated until direct genetic testing became available in the late 1960s[68]. Thus, the proceeding discussion will review current knowledge on this debilitating condition and evaluate the recent developments in treatment approaches.

As the most common inherited ataxia—defined as the compromised coordination of voluntary muscle movement—FRDA is an autosomal recessive, neurodegenerative disorder involving multiple organ systems including the central and peripheral nervous systems and the cardiovascular system[68,69]. At the molecular level, a gene, X25, encodes a 210-amino acid protein known as "frataxin", or FXN, and has been identified as a critical region susceptible to mutation for the FRDA locus on chromosome 9q13[69]. Specifically, the most common defect leading to the development of FRDA is due to a large, homozygous intronic expansion of guanine–adenine–adenine (GAA) trinucleotide repeats in intron 1 of the said frataxin gene[70,71]. Frataxin, a highly conserved small mitochondrial protein, is required for efficient regulation and homeostasis of cellular iron stores[72]. Consequently, the dysregulation of this critical molecule's function is associated with mitochondrial iron overload, iron-sulfur cluster biosynthesis, and free radical oxidative stress[73,74]. A graphical representation of the molecular etiology and subsequent biological consequences of dysfunctional frataxin protein function can be seen in Figure 3 below.

Clinically, due to frataxin's ubiquitous biochemical role in numerous cellular pathways, humans with frataxin deficiency manifest with dysfunction in the central and peripheral nervous system, heart, skeleton, and even endocrine organs including the pancreas[71]. Regarding FRDA in particular, many of these patients classically present with neuropathologic disabilities including progressive ataxia, peripheral sensory loss, and muscle weakness beginning between the ages of 5 and 15 years old[76]. The aforementioned manifestations are chiefly secondary to neuropathy in the dorsal root ganglia, accompanied by degeneration of both peripheral sensory nerve fibers and posterior columns of the spinal cord[76]. Moreover, non-neurological areas of morbidity include the heart, typically in the form of left ventricular hypertrophy due to mitochondrial proliferation, and the pancreas, with approximately 10% of all FRDA patients developing diabetes mellitus[76,77].



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Figure 3 Frataxin protein dysregulation. The 2100 amino acid protein frataxin is encoded within the first intron of the *FXN* gene on chromosome 9q13. Due to trinucleotide repeat expansions ranging from approximately 44–1700 "GAA" triplet sequences, affected individuals experience numerous characteristic signs and symptoms of Friedreich Ataxia. At the cellular level, dysregulation of this small mitochondrial protein results in the overproduction of iron-sulfur clusters, free radical oxidative stress, and mitochondrial iron overload[75]. FXN: Frataxin; ROS: Reactive oxygen species.

Before the dawn of genetic testing, the constellation of the above-mentioned signs and symptoms as well as MRI was employed in the diagnosis of FRDA[78]. Presently, however, this neurologic disorder is more precisely diagnosed using modern genetic testing such as Southern blot and conventional polymerase chain reaction (PCR) techniques[79]. The accurate diagnosis of FRDA helps in differentiating it from other ataxias and provides a guide for physicians to appropriately tackle the treatment and management of this progressive disorder. Despite the heterogeneity of symptomatic presentation, most patients will lose the ability to independently walk, stand, and/or sit within 10-15 years of initial disease onset[76]. Therefore, there is a persisting call for advancements in techniques and approaches for treating FRDA.

In the past, there were no effective protective pharmacological agents for FRDA, and thus treatment was aimed at symptomatic management and physical therapy for impaired motor function[80]. Further, aggressive surveillance was and still is used in managing progressive cardiomyopathy, arrhythmias, and diabetes mellitus to improve the quality of life for these patients[80,81]. More recently, treatment approaches to address the mitochondrial dysfunction caused by a mutation to the frataxin protein include mitochondrial function enhancers, free radical scavengers, and iron chelators such as coenzyme Q_{10} and its synthetic analog idebenone, as well as vitamin E[82]. Although the employment of such molecules is mechanistically sound, the pre-clinical and clinical trial data on their use has demonstrated little to no therapeutic effect across multiple studies and even worsened enzymatic activity[80]. As of February 2023, however, the first FDA-approved treatment for FRDA became available within the United States. Developed by Reata Pharmaceuticals, Inc., the small molecule Omaveloxolone, or SKYCLARYS™, is an orally active drug aimed at alleviating oxidative stress, mitochondrial damage and dysfunction as a direct result of Nrf2 pathway suppression found in Friedreich patients[83]. As a novel pharmaceutical with early proven safety and effectiveness, Omaveloxolone is a promising emerging option for FRDA treatment.

In addition to symptomatic treatment and management, newer therapeutics for neurological disorders are being developed, evidenced by the rapid increase in research surrounding gene and cell therapy for FRDA between the years 2000 and the present day. Considering the severity of neurophysiological abnormalities is strongly correlated with the size of the GAA repeat expansion implicated in the genetic inheritance of FRDA, many of these novel techniques are aimed at altering this pattern. Specifically, the use of the CRISPR-Cas9 system in GAA expansion-based animal models such as YG8-derived cells and mouse models demonstrated promising results in the successful editing of GAA expansion both *in vitro* and *in vivo*[84]. Contrastingly, instead of altering the primary genetic mutation, other more recent methods of ataxia reversal are aimed at inducing the expression of the frataxin protein itself[85]. Particularly, Piguet *et al*[86] demonstrated the complete reversal of sensory ataxia and cardiomyopathy *via* viral vector-based introduction of AAV-expressing frataxin (AAV-FXN) in cells and mouse models. Finally, other prospective therapeutics look to stimulate the direct transcription of FXN and/or increase FXN mRNA stability *via* interferon administration, or indirectly *via* the stimulation of NRF2—a transcription factor whose levels are intimately associated with FXN mRNA expression[87].

Altogether, these animal-based models hold promising treatment options for future application to clinical medicine, for now, more research is necessary before translation into FRDA human-based clinical trials.

INHIBITED COGNITIVE DEVELOPMENT

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are the first known examples of diseases relative to imprinted genes. These diseases have their own clinically distinct behavioral, cognitive, and neurologic phenotypes. However, they are still considered "sister disorders" due to the common origin of the disease from the imprint gene abnormalities in the 15q11-q13 region[88]. The difference in the diseases is that with PWS, the contribution is paternal whereas with AS, the contribution is maternal. This parent-of-origin difference is the reason for the variable expression of the disease depending on the sex of the parent from whom the disease is inherited[88,89].

PWS

PWS is suspected in individuals that present with clinical features of hypotonia (during the first few years) that lead to hyperphagia, hypogonadism, short stature, and mild mental retardation[90]. However, regarding the 15q11-q13 region, the abnormality is suggestive of PWS, but not diagnostic. The diagnosis of PWS is established by identifying the abnormal DNA-methylation and maternal-allele imprinting in the Prader-Willi critical region. This can be due to either the deletion of the paternal allele, maternal disomy, and/or imprinting defect causing the absence of the paternal allele [91]. Moreover, point mutation does not cause PWS because it is a factor of multiple gene products. An exception to this rule is when the point mutation is seen in the imprinting control region[92]. In these cases, although very rare, the loss of function has been seen to contribute to many different aspects of the PWS phenotype[93].

The underlying molecular defect in PWS further opens a great scope of molecular treatment and epigenetic therapy. An increasing number of studies are pointing toward gene SNORD116 being the main causative player in PWS[94-96]. Deactivation of the SNORD116 gene is associated with a complex of a zinc finger protein ZNF274 and SETDB1 a histone H3 methyltransferase[97-99]. Thus, CRISPR-Cas systems can be employed to deactivate ZNF274 and/or SETDB1 and increase the expression of SNORD116. One of the drawbacks to this approach is that the SETDB1 is not specific and thus, targeting it can have many off-label systemic effects.

Another approach explored in many studies was to inhibit G9a, another histone H3 methyltransferase, which restored the targeted genes from the silenced maternal chromosome[100-102]. Based on these studies, the use of a G9a inhibitor improved perinatal lethality and poor growth, which ultimately can improve the life span[100-102]. But again, one of the drawbacks of these research studies is that they are all performed with either mouse models or PWS patient-derived cells. Thus, the efficacy of gene therapy in PWS patients is yet to be explored and proven to be beneficial. Moreover, during the development of new drug therapies, focus should be put on off-target effects. Epigenetic drugs are known to have complex and broad effects, and thus, the development of specific molecular therapy for PWS patients is necessary.

AS

AS clinically presents as severe developmental delay, minimal or no speech, difficulty walking, and a unique behavioral phenotype that includes frequent smiling and excessive laughter[103]. As mentioned above, defects in the 15q11.2-q13 chromosome region are the genetic basis of both PWS and AS. In terms of AS however, the maternally expressed allele does not produce functional gene product, and the paternal allele is imprinted. The gene of interest in terms of AS is the UBE3A sequence which is mutated in certain individuals with AS[103].

In terms of epigenetic therapy, restoration of the mutated UBE3A gene expression is more favorable and plausible rather than targeting known activities of the molecule. However, there haven't been any successful ways researched to do so. Thus, expressing the silenced paternal UBE3A has been an adequate alternative. The most common way proposed is by administering topoisomerase-I inhibitors and reactivating the inhibition of paternal UBE3A.

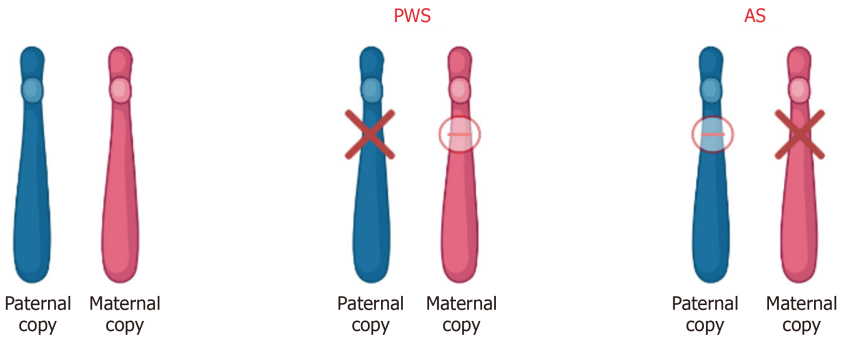
Topoisomerase-I inhibitors like topotecan and irinotecan are FDA-approved chemotherapeutic drugs. Moreover, these drugs are also shown to promote the expression of paternal UBE3A in a dose-dependent manner. The drugs work by reactivating the gene *via* reduced transcription of an imprinted antisense RNA[104-106]. Despite proving their potential, topoisomerase inhibitors are not currently used as a first-line treatment for AS due to their side effects. According to one study, topoisomerase inhibitors also reduced the levels of other genes that are linked to autism, thus increasing the potential of autism characteristics in individuals[107].

Thus, as discussed, all the approved therapies for PWS and AS target the management of symptoms rather than the actual disease. Thus, there is currently no cure for either AS or PWS. Gene and molecular therapies offer a promising way into precision medicine and the development of novel therapies however a lot more research needs to be done regarding offsetting the systemic side effects. A summary of the chromosome 15 abnormalities in PWS and AS is shown in Figure 4 below.

NEURON DETERIORATION

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder affecting both the upper and lower motor neurons and is the most common motor neuron disease in adults[108-110]. The onset of ALS symptoms typically occurs between



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Figure 4 Chromosome 15 (15q11-13) abnormality in patients with Prader-Willi syndrome and Angelman syndrome. In patients with Prader-Willi syndrome (PWS) and Angelman syndrome (AS), the imprinted gene abnormality is the 15q11-q13 region of chromosome 15. Although the abnormality is in the same region, the difference in disease and phenotype depends on the parental contribution. In PWS, the disease results due to loss of paternal gene expression. In AS, the disease results due to loss of maternal gene expression. PWS: Prader-Willi syndrome; AS: Angelman syndrome.

50-70 years old and is more common in males than females[111-113]. While some individuals can survive over 10 years with ALS, most survive less than 3 years due to the rapid disease progression[114]. Current treatments focus on the treatment of symptoms; however, their effect is often minimal by extending a patient's survival by only three months [112]. Although the etiology of ALS is currently unknown, there does appear to be a genetic component as nearly 10 percent of diagnosed individuals reported having a family member with ALS and are said to have familial ALS (fALS), while the remaining individuals are classified as having sporadic ALS (sALS)[115].

In recent years, the number of genetic mutations linked to ALS has increased. Superoxide dismutase-1 (SOD1) was the first gene discovered to be associated with ALS, and mutations of the SOD1 gene account for 10%-15% of fALS and 1%-2% of sALS[110,115-117]. More recently, mutations to the C90RF72 gene have been associated with 30%-40% of fALS and nearly 6% of sALS[115,116]. Mutations to other genes such as TDP-43, FUS, OPTN, TBK, GRN, NEK1, and C21ORF2 have also been linked to ALS[115,116]. Current evidence suggests that many of these mutations result in the aggregation of misfolded proteins, which then causes ALS[110,112]. Furthermore, knockout models tested on mice have shown that the removal of these genes typically does not result in symptoms of ALS, suggesting that these mutations result in a gain of function[112,116,117].

Current ALS treatments in clinical trials focus on gene therapy as opposed to treatment of the symptoms of ALS. These treatments attempt to replace the mutated gene, inactivate the mutated gene, or introduce a new gene to fight ALS[117]. Tofersen, an ASO, is currently in clinical trials for individuals suffering from ALS because of a SOD1 mutation. Tofersen causes the degradation of SOD1 mRNA, decreasing the amount of SOD1 protein[118]. However, ASOs such as Tofersen are unable to cross the blood-brain barrier and must be injected into the cerebrospinal fluid[117,118]. This can cause many unwanted side effects, such as inflammation, infection, and long and repeated injections[117]. As a result, nanoparticles, such as calcium phosphate lipid nanoparticles, are being developed to reduce the need for direct injection of ASOs into the CSF[117]. Additional research is being conducted on AAV vectors for the modification of genes. The major advantage of AAV vectors is that only one injection is necessary[116]. AAV vectors have the potential to deliver gene-silencing material, such as antisense sequences, to mutated genes such as the SOD1 gene[116]. However, immunoreactivity to AAV has been documented in both animal and human studies presenting future challenges to the use of AAV vectors for ALS treatment[116].

Huntington's disease

Huntington's disease (HD) is a neurodegenerative disorder resulting from the expansion of a CAG trinucleotide sequence on the *HTT* gene which encodes the huntingtin protein[119-121]. For an individual to be at risk for HD, they must have greater than 36 glutamine repeats, and as the number of repeats increases the age of onset typically decreases[120]. HD is inherited in an autosomal dominant manner, with symptoms first appearing in mid-life[121,122]. Individuals with HD experience cognitive, motor, and psychiatric symptoms that are progressive over time[119]. Current treatments for HD focus on treating symptoms but often have limited benefits, and there is no available disease-modifying treatment[121, 123].

The HTT gene encodes the huntingtin protein, which is involved in a variety of cellular functions including cell division, vesicle transportation, and transcription regulation[120]. Repeat CAG trinucleotide sequences found on exon 1 of the HTT gene cause the formation of mutant huntingtin protein aggregates within neurons[124,125]. Individuals who possess 60 or more CAG repeats will develop HD before the age of 20[124,125]. However, the CAG repeat length explains less than 50% of the HD age of onset[124]. Other factors such as glutamic acid polymorphisms and glutamate and N-methyl-D-aspartate receptor polymorphisms also play a role in the onset of HD[124]. These genetic modifiers all present potential targets for genetic treatment for HD.

As a result of the many functions of the huntingtin protein, the embryonic knockout of the *HTT* gene in mice was lethal [120,121]. Furthermore, the inactivation of the HTT gene after birth has been linked to neurological deterioration in mice [121]. Because of this, many HD disease-modifying treatments being researched focus on gene editing as opposed to gene silencing[125]. In a phase 1-2a clinical trial conducted between 2015-2017, an ASO known as IONIS-HTT$_{Rx}$ was studied for

its safety, ability to remain in the CSF, and ability to reduce mutant HTT in the CSF[126]. Upon completion of this trial, it was found that IONIS-HTT_{Rx} did not increase the number of adverse effects and resulted in a dose-dependent reduction of the mutant HTT concentration[126]. However, phase three trials show that IONIS-HTT_{Rx} may cause worse motor and cognitive function[127]. Along with ASOs, RNA interference (RNAi) technology is being developed to treat HD. RNAi technology attempts to decrease the amount of mutant HTT being translated using small non-coding RNAs[127]. This RNAi technology is typically delivered through an AAV. However, this technology is in the early stages of development, with significant testing still needed.

TUMORS

Neurofibromatosis-1

Neurofibromatosis-1 (NF-1), also known as Von Recklinghausen disease, is an autosomal dominant disorder characterized by changes in the nervous system, bones, and skin[128]. Risks associated with NF-1 include bone abnormalities, vasculopathy, and cognitive impairment[128]. NF-1 is also the common type of hamartoma neoplastic syndrome, which is the formation of benign tumorlike malformations consisting of abnormal cells and tissues[128]. Neurofibromatosis also consists of neurofibromatosis type 2 (NF-2) and Schwannmatosis[128]. NF-2 presents with similar cutaneous manifestations as NF-1 but mainly exhibits schwannomas (tumors of the nervous system), meningiomas (tumors of the meninges), and ependymomas (brain and spinal cord tumors)[129]. Of the three kinds of NF, NF-1 accounts for 96% of all cases, NF-2 accounts for 3% and Schwannomatosis accounts for less than 1%[130].

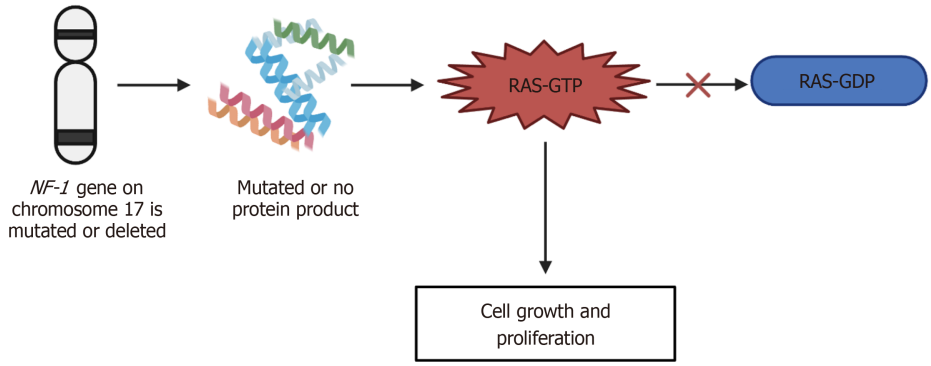
NF-1 is known as an autosomal dominant disorder that affects 1 in 2600 to 3000 individuals. All generations are included. The expression of this disease differs between individuals of the same family and from one affected family to another[128]. The NF-1 gene is located on chromosome 17 and is a tumor suppressor gene. The NF-1 gene produces a product called neurofibromin, which catalyzes the hydrolysis of guanosine triphosphate (GTP)-bound Ras to guanosine diphosphate-bound Ras, ultimately inactivating Ras GTPase[131]. Neurofibromin is expressed in various tissues throughout the body and the function of NF-1 is to modulate the activity of the RAS pathway[132]. This pathway in turn delivers signals from the granulocyte-monocyte colony-stimulating factor to proliferating cells[132]. In turn, the NF-1 protein promotes the conversion of the activated complex, Ras-GTP, to Ras-GDP, the inactivated form[133]. When the NF-1 gene is mutated or deleted, the typical phenotype of NF-1 results due to activation of the Ras-GTP, ultimately resulting in cell growth and prolferation[128]. With this disorder, penetrance is complete[134]. NF-1 has a high degree of variability in clinical presentation, which may include cutaneous, bony, vascular, and cognitive features along with multiple neoplasms. Due to these manifestations overlapping with other genetic conditions, accurate diagnosis of NF1 is important for clinical care and genetic counseling[135]. The mechanism of neurofibromatosis-1 is summarized in Figure 5 below.

New gene techniques were shown to help with NF-1-related pain. A study conducted on this rare autosomal disease suggested that collapsing response mediator protein 2 (CRMP2) is a key target for therapeutic intervention[136]. Moutal *et al*[136] highlighted there is a direct connection between the amount of neurofibromin expressed and pain. CRMP2 regulates the activity of calcium channels and increases the Ca^{2+} current and release in sensory neurons[137]. Additionally, CRMP2 binds to the C-terminus of neurofibromin, suggesting a possible correlation between CRMP2 and the pain experienced by patients with NF-1[136]. In the study, they used clustered regularly interspaced short palindromic repeats of the CRISPR-associated 9 (CAS9 genome), a commonly used DNA editing device[136]. With this, the researchers created a novel rat model of NF-1-related pain[136]. The delivery guide of Cas9 nuclease plasmid was used to generate allele-specific C-terminal truncation of neurofibromin[136]. Additionally, researchers used (S)-LCM, an inactive enantiomer of the drug Vimpat to inhibit CRMP2 phosphorylation, uncoupling CRMP2 from the NF-1 protein [136]. The rats with truncation of neurofibromin showed increases in voltage-gated calcium and sodium resulting in increased nociceptor excitability and behavioral hyperalgesia[136]. As the protein CRMP2 regulates these channels and binds to the C-terminus of neurofibromin, this indicated a possible mechanism underlying NF1 pain[136]. Targeting CRMP2 phosphorylation offers a new therapeutic way to manage the pain associated with NF-1. However, future research is needed in human trials to further investigate the link between CRMP2 and the NF-1 protein.

In children with neurofibromatosis 1, low-grade gliomas are the most common tumor and often result in significant visual loss due to tumor progression along with motor deficits[138]. The current therapeutical agent used for the management of tumor progression is vincristine/carboplatin, or vinblastine, a chemotherapeutic agent[139]. Although this agent prevents tumor progression, restoration of vision or motor function is rare[140]. A new type of therapeutic approach involves the use of mTOR inhibitors[140]. When mTOR is activated, abnormal cell growth results leading to cell proliferation and the formation of new blood vessels through angiogenesis[140]. Everolimus is derived from rapamycin and inhibits angiogenesis, hypoxia-inducible factor 1, and vascular endothelial growth factor production, and ultimately prevents the proliferation of cells[141]. When Everolimus was given to patients with NF-1, the tumor progression was significantly halted and demonstrated sufficient penetration of the blood-brain barrier[141]. This offers a promising therapeutic for children with low-grade gliomas as it is available orally and has minimal toxicities. However, further researcher is needed on Everolimus in combination with other agents to determine if it is the superior therapeutic agent or if a combination offers better results.

Tuberous sclerosis

Tuberous sclerosis is an inherited autosomal dominant neurocutaneous genetic disorder[142]. This genetic disorder affects multiple systems and often presents in children with skin lesions, seizures, and hamartomas of the brain, kidney,



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Figure 5 Mechanism of neurofibromatosis-1. If the *neurofibromatosis-1* gene on chromosome 17 is either mutated or deleted, either no protein product is formed, or a mutated protein is made. This mutation or deletion then results in the inhibition of the conversion of Ras-GTP to Ras-GDP, ultimately resulting in the increased signaling of Ras-GTP and leading to cell growth and proliferation, producing the neurofibromatosis phenotype. GTP: Guanosine triphosphate; GDP: Guanosine 5'-diphosphate; NF-1: Neurofibromatosis-1.

and heart[142]. Additionally, tuberous sclerosis impacts 1 in 5500 individuals. Affected individuals may also present with developmental delays[142]. Cardiac rhabdomyomas of cortical tubers may also be present prenatally[142]. Adulthood signs show osseous, renal, or pulmonary lesions[142]. Skin lesions are found in 90% of patients of all ages whilst, hypopigmented macules are found in early childhood[142]. Ungual fibromas appear during puberty and facial angiofibromas are more commonly found towards adolescence[143].

Tuberous sclerosis disorder arises from four mutations in tuberous sclerosis complex 1 (TSC1) (9q34) and tuberous sclerosis complex 2 (TSC2) (16p13.3) genes which respectively encode hamartin and tuberin[144,145]. This disorder has been known to have a broad spectrum of mutations in both genes[144]. No regions seem more liable to mutations and the frequency is consistently higher in TSC2 rather than in TSC1[144]. 15% of patients admitted who meet clinical criteria for tuberous sclerosis demonstrated no identifiable genetic mutations[143]. The disease itself is caused by a mutation of these genes and causes dysfunction of proteins hamartin or tuberin[143]. Hamartin's role helps control the proliferation of cell growth, division, and cell size[143]. Tuberin functions to regulate cell growth and protein synthesis through the downstream inhibition of mTOR[146]. Therefore, the loss of either of these proteins can lead to the overgrowth of lesions in many vital organs[146].

Gene therapy for tuberous sclerosis type 2 was conducted on a mouse model by delivering an adeno-associated virus (AAv9) which encoded a condensed form of tuberin[147]. A mouse model of TSC2 was generated by AAV-Cre recombinase disruption of Tsc2-floxed alleles at birth, leading to a shortened lifespan (mean 58 d) and brain pathology consistent with TSC[147]. When these mice were injected intravenously on day 21 with AAV9-cTuberin, the mean survival was extended to 462 d with a reduction in brain pathology[147]. This study demonstrated the potential of treating life-threatening TSC2 Lesions with a single intravenous injection of AAV9-cTuberin[147]. Preventions for this disease will vary depending on the developmental stage of the specific individual as tuberous sclerosis has a highly variable clinical course and the prognosis may be uncertain[147]. Options pertaining to tuberous sclerosis are limited to surgery for treating symptoms of tuberous sclerosis related to the growth of hamartomas[148]. Therefore, to better understand the genetic causation for this disease, clinical trials of mammalian target of rapamycin inhibitors, including sirolimus and Everolimus have been conducted[149]. mTOR is an evolutionary conserved serine-threonine kinase that regulates cell growth and cell survival[149]. The connection between TSC and mTOR led to the clinical use of mTOR allosteric inhibitors, Sirolimus and Everolimus[149]. Both sirolimus and Everolimus inhibit mTOR selectively with similar molecular mechanisms but distinct clinical profiles[149]. Everolimus has been approved for subependymal giant cell astrocytomas and renal angiomyolipomas in TSC patients[149]. However, sirolimus is not approved for TSC and has undergone considerable investigation to treat various aspects of TSC. However, the use of sirolimus has been studied in older children and adults with TSC[150-153]. One study published in 2023 investigated the use of sirolimus in children under the age of 2 with tuberous sclerosis complex and found that sirolimus was safe to use in children[154]. The study reported that the most common adverse events due to sirolimus use included anemia, hyperlipidemia, and thrombocytosis, which were able to be managed well[154]. Despite proving its safety through this study, further research is needed into sirolimus to demonstrate safety and efficacy through larger studies and clinical trials, but it offers a promising therapeutic option for patients with tuberous sclerosis.

CONCLUSION

Neurologic orphan diseases impact less than 200000 individuals within the United States and because they're rare, diagnosis and treatment are often difficult. However, in recent years, data has suggested that the use of whole genome sequencing has increased the diagnosis of neurologic orphan diseases, allowing patients who have these conditions to be more easily identified. With new advances in technology, more research is being devoted to developing therapeutic options for patients with neurologic orphan diseases. Such advances in research include the use of AAV vectors which

have shown positive results in SMA, ALS, Friedreich Ataxia, and NF-1. Additionally, the use of CRISPR/Cas has demonstrated promising results in DMD, Dravet syndrome, Friedreich Ataxia, NF-1, and Tuberous Sclerosis. The use of therapeutics that can cross the blood-brain barrier, such as Risdiplam and Branaplam, has increased the number of treatment options available for patients with SMA. Additionally, research into mTOR inhibitors offers a promising option for patients with NF-1 and tuberous sclerosis. Although further research is needed, these treatment options can significantly impact the quality of life and survival of patients with neurologic orphan diseases.

FOOTNOTES

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Country/Territory of origin: United States

ORCID number: Ivelina P Kioutchoukova 0009-0001-7467-2382; Devon T Foster 0009-0007-9411-9051; Rajvi N Thakkar 0009-0003-2375-5078; Marco A Foreman 0000-0002-5551-7061; Brandon J Burgess 0009-0008-7203-8469; Rebecca M Toms 0009-0005-5539-9754; Eduardo E Molina Valero 0000-0002-3235-4079; Brandon Lucke-Wold 0000-0001-6577-4080.

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REFERENCES

1 **Griggs RC**, Batshaw M, Dunkle M, Gopal-Srivastava R, Kaye E, Krischer J, Nguyen T, Paulus K, Merkel PA; Rare Diseases Clinical Research Network. Clinical research for rare disease: opportunities, challenges, and solutions. *Mol Genet Metab* 2009; **96**: 20-26 [PMID: 19013090 DOI: 10.1016/j.ymgme.2008.10.003]

2 **The Lancet Neurology**. Rare diseases: maintaining momentum. *Lancet Neurol* 2022; **21**: 203 [PMID: 35182497 DOI: 10.1016/S1474-4422(22)00046-1]

3 **Murphy SM**, Puwanant A, Griggs RC; Consortium for Clinical Investigations of Neurological Channelopathies (CINCH) and Inherited Neuropathies Consortium (INC) Consortia of the Rare Disease Clinical Research Network. Unintended effects of orphan product designation for rare neurological diseases. *Ann Neurol* 2012; **72**: 481-490 [PMID: 23109143 DOI: 10.1002/ana.23672]

4 **Stephenson D**, Ollivier C, Brinton R, Barrett J. Can Innovative Trial Designs in Orphan Diseases Drive Advancement of Treatments for Common Neurological Diseases? *Clin Pharmacol Ther* 2022; **111**: 799-806 [PMID: 35034352 DOI: 10.1002/cpt.2528]

5 **Dowden H**, Munro J. Trends in clinical success rates and therapeutic focus. *Nat Rev Drug Discov* 2019; **18**: 495-496 [PMID: 31267067 DOI: 10.1038/d41573-019-00074-z]

6 **Vinci L**. New Report Shows 28% Increase in YoY Funding for Rare Disease Drug Development in 2021. January 13, 2022. Available from: https://www.businesswire.com/news/home/20220112005794/en/New-Report-Shows-28-Increase-in-YoY-Funding-for-Rare-Disease-Drug-Development-in-2021

7 **Ibañez K**, Polke J, Hagelstrom RT, Dolzhenko E, Pasko D, Thomas ERA, Daugherty LC, Kasperaviciute D, Smith KR; WGS for Neurological Diseases Group, Deans ZC, Hill S, Fowler T, Scott RH, Hardy J, Chinnery PF, Houlden H, Rendon A, Caulfield MJ, Eberle MA, Taft RJ, Tucci A; Genomics England Research Consortium. Whole genome sequencing for the diagnosis of neurological repeat expansion disorders in the UK: a retrospective diagnostic accuracy and prospective clinical validation study. *Lancet Neurol* 2022; **21**: 234-245 [PMID: 35182509 DOI: 10.1016/S1474-4422(21)00462-2]

8 **Redman M**, King A, Watson C, King D. What is CRISPR/Cas9? *Arch Dis Child Educ Pract Ed* 2016; **101**: 213-215 [PMID: 27059283 DOI: 10.1136/archdischild-2016-310459]

9 **Hill SF**, Meisler MH. Antisense Oligonucleotide Therapy for Neurodevelopmental Disorders. *Dev Neurosci* 2021; **43**: 247-252 [PMID: 34412058 DOI: 10.1159/000517686]

10 **Naso MF**, Tomkowicz B, Perry WL 3rd, Strohl WR. Adeno-Associated Virus (AAV) as a Vector for Gene Therapy. *BioDrugs* 2017; **31**: 317-334 [PMID: 28669112 DOI: 10.1007/s40259-017-0234-5]

11 **Wang D**, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat Rev Drug Discov* 2019; **18**: 358-378 [PMID: 30710128 DOI: 10.1038/s41573-019-0012-9]

12 **Xu T**, Sun D, Chen Y, Ouyang L. Targeting mTOR for fighting diseases: A revisited review of mTOR inhibitors. *Eur J Med Chem* 2020; **199**: 112391 [PMID: 32416459 DOI: 10.1016/j.ejmech.2020.112391]

13 **Ross LF**, Kwon JM. Spinal Muscular Atrophy: Past, Present, and Future. *Neoreviews* 2019; **20**: e437-e451 [PMID: 31371553 DOI: 10.1542/neo.20-8-e437]

14 **Lefebvre S**, Bürglen L, Reboullet S, Clermont O, Burlet P, Viollet L, Benichou B, Cruaud C, Millasseau P, Zeviani M. Identification and

characterization of a spinal muscular atrophy-determining gene. *Cell* 1995; **80**: 155-165 [PMID: 7813012 DOI: 10.1016/0092-8674(95)90460-3]

15 **D'Amico A**, Mercuri E, Tiziano FD, Bertini E. Spinal muscular atrophy. *Orphanet J Rare Dis* 2011; **6**: 71 [PMID: 22047105 DOI: 10.1186/1750-1172-6-71]

16 **Prior TW**, Leach ME, Finanger E. Spinal Muscular Atrophy. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJ, Gripp KW, Amemiya A, editors. GeneReviews®. Seattle (WA): University of Washington, Seattle, 1993. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1352/

17 **Schorling DC**, Pechmann A, Kirschner J. Advances in Treatment of Spinal Muscular Atrophy - New Phenotypes, New Challenges, New Implications for Care. *J Neuromuscul Dis* 2020; **7**: 1-13 [PMID: 31707373 DOI: 10.3233/JND-190424]

18 **Singh NK**, Singh NN, Androphy EJ, Singh RN. Splicing of a critical exon of human Survival Motor Neuron is regulated by a unique silencer element located in the last intron. *Mol Cell Biol* 2006; **26**: 1333-1346 [PMID: 16449646 DOI: 10.1128/MCB.26.4.1333-1346.2006]

19 **Rigo F**, Hua Y, Krainer AR, Bennett CF. Antisense-based therapy for the treatment of spinal muscular atrophy. *J Cell Biol* 2012; **199**: 21-25 [PMID: 23027901 DOI: 10.1083/jcb.201207087]

20 **Passini MA**, Bu J, Richards AM, Kinnecom C, Sardi SP, Stanek LM, Hua Y, Rigo F, Matson J, Hung G, Kaye EM, Shihabuddin LS, Krainer AR, Bennett CF, Cheng SH. Antisense oligonucleotides delivered to the mouse CNS ameliorate symptoms of severe spinal muscular atrophy. *Sci Transl Med* 2011; **3**: 72ra18 [PMID: 21368223 DOI: 10.1126/scitranslmed.3001777]

21 **Finkel RS**, Chiriboga CA, Vajsar J, Day JW, Montes J, De Vivo DC, Yamashita M, Rigo F, Hung G, Schneider E, Norris DA, Xia S, Bennett CF, Bishop KM. Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study. *Lancet* 2016; **388**: 3017-3026 [PMID: 27939059 DOI: 10.1016/S0140-6736(16)31408-8]

22 **Finkel RS**, Mercuri E, Darras BT, Connolly AM, Kuntz NL, Kirschner J, Chiriboga CA, Saito K, Servais L, Tizzano E, Topaloglu H, Tulinius M, Montes J, Glanzman AM, Bishop K, Zhong ZJ, Gheuens S, Bennett CF, Schneider E, Farwell W, De Vivo DC; ENDEAR Study Group. Nusinersen *vs* Sham Control in Infantile-Onset Spinal Muscular Atrophy. *N Engl J Med* 2017; **377**: 1723-1732 [PMID: 29091570 DOI: 10.1056/NEJMoa1702752]

23 **Mercuri E**, Darras BT, Chiriboga CA, Day JW, Campbell C, Connolly AM, Iannaccone ST, Kirschner J, Kuntz NL, Saito K, Shieh PB, Tulinius M, Mazzone ES, Montes J, Bishop KM, Yang Q, Foster R, Gheuens S, Bennett CF, Farwell W, Schneider E, De Vivo DC, Finkel RS; CHERISH Study Group. Nusinersen *vs* Sham Control in Later-Onset Spinal Muscular Atrophy. *N Engl J Med* 2018; **378**: 625-635 [PMID: 29443664 DOI: 10.1056/NEJMoa1710504]

24 **Poirier A**, Weetall M, Heinig K, Bucheli F, Schoenlein K, Alsenz J, Bassett S, Ullah M, Senn C, Ratni H, Naryshkin N, Paushkin S, Mueller L. Risdiplam distributes and increases SMN protein in both the central nervous system and peripheral organs. *Pharmacol Res Perspect* 2018; **6**: e00447 [PMID: 30519476 DOI: 10.1002/prp2.447]

25 **Dayton RD**, Wang DB, Klein RL. The advent of AAV9 expands applications for brain and spinal cord gene delivery. *Expert Opin Biol Ther* 2012; **12**: 757-766 [PMID: 22519910 DOI: 10.1517/14712598.2012.681463]

26 **Valori CF**, Ning K, Wyles M, Mead RJ, Grierson AJ, Shaw PJ, Azzouz M. Systemic delivery of scAAV9 expressing SMN prolongs survival in a model of spinal muscular atrophy. *Sci Transl Med* 2010; **2**: 35ra42 [PMID: 20538619 DOI: 10.1126/scitranslmed.3000830]

27 **Blair HA**. Onasemnogene Abeparvovec: A Review in Spinal Muscular Atrophy. *CNS Drugs* 2022; **36**: 995-1005 [PMID: 35960489 DOI: 10.1007/s40263-022-00941-1]

28 **Al-Zaidy SA**, Kolb SJ, Lowes L, Alfano LN, Shell R, Church KR, Nagendran S, Sproule DM, Feltner DE, Wells C, Ogrinc F, Menier M, L'Italien J, Arnold WD, Kissel JT, Kaspar BK, Mendell JR. AVXS-101 (Onasemnogene Abeparvovec) for SMA1: Comparative Study with a Prospective Natural History Cohort. *J Neuromuscul Dis* 2019; **6**: 307-317 [PMID: 31381526 DOI: 10.3233/JND-190403]

29 **Mercuri E**, Sumner CJ, Muntoni F, Darras BT, Finkel RS. Spinal muscular atrophy. *Nat Rev Dis Primers* 2022; **8**: 52 [PMID: 35927425 DOI: 10.1038/s41572-022-00380-8]

30 **Duan D**, Goemans N, Takeda S, Mercuri E, Aartsma-Rus A. Duchenne muscular dystrophy. *Nat Rev Dis Primers* 2021; **7**: 13 [PMID: 33602943 DOI: 10.1038/s41572-021-00248-3]

31 **Venugopal V**, Pavlakis S. Duchenne Muscular Dystrophy. In: StatPearls. Treasure Island (FL): StatPearls Publishing, 2023. Available from: http://www.ncbi.nlm.nih.gov/books/NBK482346/

32 **Falzarano MS**, Scotton C, Passarelli C, Ferlini A. Duchenne Muscular Dystrophy: From Diagnosis to Therapy. *Molecules* 2015; **20**: 18168-18184 [PMID: 26457695 DOI: 10.3390/molecules201018168]

33 **Sun C**, Shen L, Zhang Z, Xie X. Therapeutic Strategies for Duchenne Muscular Dystrophy: An Update. *Genes (Basel)* 2020; **11** [PMID: 32717791 DOI: 10.3390/genes11080837]

34 **Ciafaloni E**, Moxley RT. Treatment options for Duchenne muscular dystrophy. *Curr Treat Options Neurol* 2008; **10**: 86-93 [PMID: 18334131 DOI: 10.1007/s11940-008-0010-4]

35 **Ortez C**, Natera de Benito D, Carrera García L, Expósito J, Nolasco G, Nascimento A. [Advances in the treatment of Duchenne muscular dystrophy]. *Medicina (B Aires)* 2019; **79** Suppl 3: 77-81 [PMID: 31603849]

36 **Aartsma-Rus A**, Straub V, Hemmings R, Haas M, Schlosser-Weber G, Stoyanova-Beninska V, Mercuri E, Muntoni F, Sepodes B, Vroom E, Balabanov P. Development of Exon Skipping Therapies for Duchenne Muscular Dystrophy: A Critical Review and a Perspective on the Outstanding Issues. *Nucleic Acid Ther* 2017; **27**: 251-259 [PMID: 28796573 DOI: 10.1089/nat.2017.0682]

37 **Wong TWY**, Cohn RD. Therapeutic Applications of CRISPR/Cas for Duchenne Muscular Dystrophy. *Curr Gene Ther* 2017; **17**: 301-308 [PMID: 29173172 DOI: 10.2174/1566523217666171121165046]

38 **Wang JZ**, Wu P, Shi ZM, Xu YL, Liu ZJ. The AAV-mediated and RNA-guided CRISPR/Cas9 system for gene therapy of DMD and BMD. *Brain Dev* 2017; **39**: 547-556 [PMID: 28390761 DOI: 10.1016/j.braindev.2017.03.024]

39 **Rodgers BD**, Bishaw Y, Kagel D, Ramos JN, Maricelli JW. Micro-dystrophin Gene Therapy Partially Enhances Exercise Capacity in Older Adult mdx Mice. *Mol Ther Methods Clin Dev* 2020; **17**: 122-132 [PMID: 31909085 DOI: 10.1016/j.omtm.2019.11.015]

40 **Duan D**. Systemic AAV Micro-dystrophin Gene Therapy for Duchenne Muscular Dystrophy. *Mol Ther* 2018; **26**: 2337-2356 [PMID: 30093306 DOI: 10.1016/j.ymthe.2018.07.011]

41 **Lazaridis D**, Eraikhuemen N, Williams K, Lovince J. Treatment of Seizures Associated with Lennox-Gastaut and Dravet Syndromes: A Focus on Cannabidiol Oral Solution. *P T* 2019; **44**: 255-266 [PMID: 31080333]

42 **Scheffer IE**, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, Hirsch E, Jain S, Mathern GW, Moshé SL, Nordli DR, Perucca E, Tomson T, Wiebe S, Zhang YH, Zuberi SM. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. *Epilepsia* 2017; **58**: 512-521 [PMID: 28276062 DOI: 10.1111/epi.13709]

43 **Li W**, Schneider AL, Scheffer IE. Defining Dravet syndrome: An essential pre-requisite for precision medicine trials. *Epilepsia* 2021; **62**: 2205-2217 [PMID: 34338318 DOI: 10.1111/epi.17015]

44 **Berg AT**, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, Engel J, French J, Glauser TA, Mathern GW, Moshé SL, Nordli D, Plouin P, Scheffer IE. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. *Epilepsia* 2010; **51**: 676-685 [PMID: 20196795 DOI: 10.1111/j.1528-1167.2010.02522.x]

45 **Wirrell EC**, Laux L, Donner E, Jette N, Knupp K, Meskis MA, Miller I, Sullivan J, Welborn M, Berg AT. Optimizing the Diagnosis and Management of Dravet Syndrome: Recommendations From a North American Consensus Panel. *Pediatr Neurol* 2017; **68**: 18-34.e3 [PMID: 28284397 DOI: 10.1016/j.pediatrneurol.2017.01.025]

46 **Claes L**, Del-Favero J, Ceulemans B, Lagae L, Van Broeckhoven C, De Jonghe P. De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. *Am J Hum Genet* 2001; **68**: 1327-1332 [PMID: 11359211 DOI: 10.1086/320609]

47 **Tahara M**, Higurashi N, Hata J, Nishikawa M, Ito K, Hirose S, Kaneko T, Mashimo T, Sakuma T, Yamamoto T, Okano HJ. Developmental changes in brain activity of heterozygous Scn1a knockout rats. *Front Neurol* 2023; **14**: 1125089 [PMID: 36998780 DOI: 10.3389/fneur.2023.1125089]

48 **He Z**, Li Y, Zhao X, Li B. Dravet syndrome: Advances in etiology, clinical presentation, and treatment. *Epilepsy Res* 2022; **188**: 107041 [PMID: 36368227 DOI: 10.1016/j.eplepsyres.2022.107041]

49 **Han Z**, Chen C, Christiansen A, Ji S, Lin Q, Anumonwo C, Liu C, Leiser SC, Meena, Aznarez I, Liau G, Isom LL. Antisense oligonucleotides increase Scn1a expression and reduce seizures and SUDEP incidence in a mouse model of Dravet syndrome. *Sci Transl Med* 2020; **12** [PMID: 32848094 DOI: 10.1126/scitranslmed.aaz6100]

50 **Higurashi N**, Broccoli V, Hirose S. Genetics and gene therapy in Dravet syndrome. *Epilepsy Behav* 2022; **131**: 108043 [PMID: 34053869 DOI: 10.1016/j.yebeh.2021.108043]

51 **Colasante G**, Lignani G, Brusco S, Di Berardino C, Carpenter J, Giannelli S, Valassina N, Bido S, Ricci R, Castoldi V, Marenna S, Church T, Massimino L, Morabito G, Benfenati F, Schorge S, Leocani L, Kullmann DM, Broccoli V. dCas9-Based Scn1a Gene Activation Restores Inhibitory Interneuron Excitability and Attenuates Seizures in Dravet Syndrome Mice. *Mol Ther* 2020; **28**: 235-253 [PMID: 31607539 DOI: 10.1016/j.ymthe.2019.08.018]

52 **Ohtahara S**, Ohtsuka Y, Yamatogi Y, Oka E. The early-infantile epileptic encephalopathy with suppression-burst: developmental aspects. *Brain Dev* 1987; **9**: 371-376 [PMID: 3434712 DOI: 10.1016/s0387-7604(87)80110-9]

53 **Radaelli G**, de Souza Santos F, Borelli WV, Pisani L, Nunes ML, Scorza FA, da Costa JC. Causes of mortality in early infantile epileptic encephalopathy: A systematic review. *Epilepsy Behav* 2018; **85**: 32-36 [PMID: 29906699 DOI: 10.1016/j.yebeh.2018.05.015]

54 **Symonds JD**, Elliott KS, Shetty J, Armstrong M, Brunklaus A, Cutcutache I, Diver LA, Dorris L, Gardiner S, Jollands A, Joss S, Kirkpatrick M, McLellan A, MacLeod S, O'Regan M, Page M, Pilley E, Pilz DT, Stephen E, Stewart K, Ashrafian H, Knight JC, Zuberi SM. Early childhood epilepsies: epidemiology, classification, aetiology, and socio-economic determinants. *Brain* 2021; **144**: 2879-2891 [PMID: 34687210 DOI: 10.1093/brain/awab162]

55 **Cossu A**, Santos JL, Galati G, Nikanorova M, Costa P, Mang Y, Silahtaroglu A, Rubboli G, Tommerup N, Dalla Bernardina B, Møller RS, Cantalupo G, Gardella E. PRRT2 benign familial infantile seizures (BFIS) with atypical evolution to encephalopathy related to status epilepticus during sleep (ESES). *Neurol Sci* 2023; **44**: 2173-2176 [PMID: 36913149 DOI: 10.1007/s10072-023-06735-7]

56 **Pisano T**, Numis AL, Heavin SB, Weckhuysen S, Angriman M, Suls A, Podesta B, Thibert RL, Shapiro KA, Guerrini R, Scheffer IE, Marini C, Cilio MR. Early and effective treatment of KCNQ2 encephalopathy. *Epilepsia* 2015; **56**: 685-691 [PMID: 25880994 DOI: 10.1111/epi.12984]

57 **Wolff M**, Johannesen KM, Hedrich UBS, Masnada S, Rubboli G, Gardella E, Lesca G, Ville D, Milh M, Villard L, Afenjar A, Chantot-Bastaraud S, Mignot C, Lardennois C, Nava C, Schwarz N, Gérard M, Perrin L, Doummar D, Auvin S, Miranda MJ, Hempel M, Brilstra E, Knoers N, Verbeek N, van Kempen M, Braun KP, Mancini G, Biskup S, Hörtnagel K, Döcker M, Bast T, Loddenkemper T, Wong-Kisiel L, Baumeister FM, Fazeli W, Striano P, Dilena R, Fontana E, Zara F, Kurlemann G, Klepper J, Thoene JG, Arndt DH, Deconinck N, Schmitt-Mechelke T, Maier O, Muhle H, Wical B, Finetti C, Brückner R, Pietz J, Golla G, Jillella D, Linnet KM, Charles P, Moog U, Õiglane-Shlik E, Mantovani JF, Park K, Deprez M, Lederer D, Mary S, Scalais E, Selim L, Van Coster R, Lagae L, Nikanorova M, Hjalgrim H, Korenke GC, Trivisano M, Specchio N, Ceulemans B, Dorn T, Helbig KL, Hardies K, Stamberger H, de Jonghe P, Weckhuysen S, Lemke JR, Krägeloh-Mann I, Helbig I, Kluger G, Lerche H, Møller RS. Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders. *Brain* 2017; **140**: 1316-1336 [PMID: 28379373 DOI: 10.1093/brain/awx054]

58 **van der Louw E**, van den Hurk D, Neal E, Leiendecker B, Fitzsimmon G, Dority L, Thompson L, Marchió M, Dudzińska M, Dressler A, Klepper J, Auvin S, Cross JH. Ketogenic diet guidelines for infants with refractory epilepsy. *Eur J Paediatr Neurol* 2016; **20**: 798-809 [PMID: 27470655 DOI: 10.1016/j.ejpn.2016.07.009]

59 **Goodspeed K**, Liu JS, Nye KL, Prasad S, Sadhu C, Tavakkoli F, Bilder DA, Minassian BA, Bailey RM. SLC13A5 Deficiency Disorder: From Genetics to Gene Therapy. *Genes (Basel)* 2022; **13** [PMID: 36140822 DOI: 10.3390/genes13091655]

60 **Mastrangelo M**. Lennox-Gastaut Syndrome: A State of the Art Review. *Neuropediatrics* 2017; **48**: 143-151 [PMID: 28346953 DOI: 10.1055/s-0037-1601324]

61 **Arzimanoglou A**, French J, Blume WT, Cross JH, Ernst JP, Feucht M, Genton P, Guerrini R, Kluger G, Pellock JM, Perucca E, Wheless JW. Lennox-Gastaut syndrome: a consensus approach on diagnosis, assessment, management, and trial methodology. *Lancet Neurol* 2009; **8**: 82-93 [PMID: 19081517 DOI: 10.1016/S1474-4422(08)70292-8]

62 **Asadi-Pooya AA**. Lennox-Gastaut syndrome: a comprehensive review. *Neurol Sci* 2018; **39**: 403-414 [PMID: 29124439 DOI: 10.1007/s10072-017-3188-y]

63 **Lemmon ME**, Terao NN, Ng YT, Reisig W, Rubenstein JE, Kossoff EH. Efficacy of the ketogenic diet in Lennox-Gastaut syndrome: a retrospective review of one institution's experience and summary of the literature. *Dev Med Child Neurol* 2012; **54**: 464-468 [PMID: 22443637 DOI: 10.1111/j.1469-8749.2012.04233.x]

64 **Knupp KG**, Scheffer IE, Ceulemans B, Sullivan JE, Nickels KC, Lagae L, Guerrini R, Zuberi SM, Nabbout R, Riney K, Shore S, Agarwal A, Lock M, Farfel GM, Galer BS, Gammaitoni AR, Davis R, Gil-Nagel A. Efficacy and Safety of Fenfluramine for the Treatment of Seizures Associated With Lennox-Gastaut Syndrome: A Randomized Clinical Trial. *JAMA Neurol* 2022; **79**: 554-564 [PMID: 35499850 DOI: 10.1001/jamaneurol.2022.0829]

65 **Cross JH**, Auvin S, Falip M, Striano P, Arzimanoglou A. Expert Opinion on the Management of Lennox-Gastaut Syndrome: Treatment Algorithms and Practical Considerations. *Front Neurol* 2017; **8**: 505 [PMID: 29085326 DOI: 10.3389/fneur.2017.00505]

66 **Pandolfo M**. Friedreich ataxia. *Arch Neurol* 2008; **65**: 1296-1303 [PMID: 18852343 DOI: 10.1001/archneur.65.10.1296]

67 **Koeppen AH**. Nikolaus Friedreich and degenerative atrophy of the dorsal columns of the spinal cord. *J Neurochem* 2013; **126** Suppl 1: 4-10 [PMID: 23859337 DOI: 10.1111/jnc.12218]

68 **Dürr A**, Cossee M, Agid Y, Campuzano V, Mignard C, Penet C, Mandel JL, Brice A, Koenig M. Clinical and genetic abnormalities in patients with Friedreich's ataxia. *N Engl J Med* 1996; **335**: 1169-1175 [PMID: 8815938 DOI: 10.1056/NEJM199610173351601]

69 **Campuzano V**, Montermini L, Moltò MD, Pianese L, Cossée M, Cavalcanti F, Monros E, Rodius F, Duclos F, Monticelli A, Zara F, Cañizares J, Koutnikova H, Bidichandani SI, Gellera C, Brice A, Trouillas P, De Michele G, Filla A, De Frutos R, Palau F, Patel PI, Di Donato S, Mandel JL, Cocozza S, Koenig M, Pandolfo M. Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science* 1996; **271**: 1423-1427 [PMID: 8596916 DOI: 10.1126/science.271.5254.1423]

70 **Pandolfo M**. Molecular pathogenesis of Friedreich ataxia. *Arch Neurol* 1999; **56**: 1201-1208 [PMID: 10520935 DOI: 10.1001/archneur.56.10.1201]

71 **Koeppen AH**. Friedreich's ataxia: pathology, pathogenesis, and molecular genetics. *J Neurol Sci* 2011; **303**: 1-12 [PMID: 21315377 DOI: 10.1016/j.jns.2011.01.010]

72 **Pastore A**, Puccio H. Frataxin: a protein in search for a function. *J Neurochem* 2013; **126** Suppl 1: 43-52 [PMID: 23859340 DOI: 10.1111/jnc.12220]

73 **Czechowska E**. [Parasitic diseases of the visual system (author's transl)]. *Klin Oczna* 1977; **47**: 407-409 [PMID: 926674]

74 **Bencze KZ**, Kondapalli KC, Cook JD, McMahon S, Millán-Pacheco C, Pastor N, Stemmler TL. The structure and function of frataxin. *Crit Rev Biochem Mol Biol* 2006; **41**: 269-291 [PMID: 16911956 DOI: 10.1080/10409230600846058]

75 **Sandi C**, Sandi M, Anjomani Virmouni S, Al-Mahdawi S, Pook MA. Epigenetic-based therapies for Friedreich ataxia. *Front Genet* 2014; **5**: 165 [PMID: 24917884 DOI: 10.3389/fgene.2014.00165]

76 **Delatycki MB**, Corben LA. Clinical features of Friedreich ataxia. *J Child Neurol* 2012; **27**: 1133-1137 [PMID: 22752493 DOI: 10.1177/0883073812448230]

77 **Hanson E**, Sheldon M, Pacheco B, Alkubeysi M, Raizada V. Heart disease in Friedreich's ataxia. *World J Cardiol* 2019; **11**: 1-12 [PMID: 30705738 DOI: 10.4330/wjc.v11.i1.1]

78 **Wessel K**, Schroth G, Diener HC, Müller-Forell W, Dichgans J. Significance of MRI-confirmed atrophy of the cranial spinal cord in Friedreich's ataxia. *Eur Arch Psychiatry Neurol Sci* 1989; **238**: 225-230 [PMID: 2759158 DOI: 10.1007/BF00381470]

79 **Muthuswamy S**, Agarwal S, Dalal A. Diagnosis and Genetic Counseling for Friedreich's Ataxia: A time for consideration of TP-PCR in an Indian Setup. Hippokratia 2013; 17: 38–41 [PMID: 23935342]

80 **Lynch DR**, Farmer JM, Balcer LJ, Wilson RB. Friedreich ataxia: effects of genetic understanding on clinical evaluation and therapy. *Arch Neurol* 2002; **59**: 743-747 [PMID: 12020255 DOI: 10.1001/archneur.59.5.743]

81 **Schulz JB**, Boesch S, Bürk K, Dürr A, Giunti P, Mariotti C, Pousset F, Schöls L, Vankan P, Pandolfo M. Diagnosis and treatment of Friedreich ataxia: a European perspective. *Nat Rev Neurol* 2009; **5**: 222-234 [PMID: 19347027 DOI: 10.1038/nrneurol.2009.26]

82 **Montenegro L**, Turnaturi R, Parenti C, Pasquinucci L. Idebenone: Novel Strategies to Improve Its Systemic and Local Efficacy. *Nanomaterials (Basel)* 2018; **8** [PMID: 29401722 DOI: 10.3390/nano8020087]

83 **Lee A**. Omaveloxolone: First Approval. *Drugs* 2023; **83**: 725-729 [PMID: 37155124 DOI: 10.1007/s40265-023-01874-9]

84 **Ouellet DL**, Cherif K, Rousseau J, Tremblay JP. Deletion of the GAA repeats from the human frataxin gene using the CRISPR-Cas9 system in YG8R-derived cells and mouse models of Friedreich ataxia. *Gene Ther* 2017; **24**: 265-274 [PMID: 28024081 DOI: 10.1038/gt.2016.89]

85 **Evans-Galea MV**, Pébay A, Dottori M, Corben LA, Ong SH, Lockhart PJ, Delatycki MB. Cell and gene therapy for Friedreich ataxia: progress to date. *Hum Gene Ther* 2014; **25**: 684-693 [PMID: 24749505 DOI: 10.1089/hum.2013.180]

86 **Piguet F**, de Montigny C, Vaucamps N, Reutenauer L, Eisenmann A, Puccio H. Rapid and Complete Reversal of Sensory Ataxia by Gene Therapy in a Novel Model of Friedreich Ataxia. *Mol Ther* 2018; **26**: 1940-1952 [PMID: 29853274 DOI: 10.1016/j.ymthe.2018.05.006]

87 **Zhang S**, Napierala M, Napierala JS. Therapeutic Prospects for Friedreich's Ataxia. *Trends Pharmacol Sci* 2019; **40**: 229-233 [PMID: 30905359 DOI: 10.1016/j.tips.2019.02.001]

88 **Cassidy SB**, Dykens E, Williams CA. Prader-Willi and Angelman syndromes: sister imprinted disorders. *Am J Med Genet* 2000; **97**: 136-146 [PMID: 11180221 DOI: 10.1002/1096-8628(200022)97:2<136::aid-ajmg5>3.0.co;2-v]

89 **Ma VK**, Mao R, Toth JN, Fulmer ML, Egense AS, Shankar SP. Prader-Willi and Angelman Syndromes: Mechanisms and Management. *Appl Clin Genet* 2023; **16**: 41-52 [PMID: 37051256 DOI: 10.2147/TACG.S372708]

90 **Vogels A**, De Hert M, Descheemaeker MJ, Govers V, Devriendt K, Legius E, Prinzie P, Fryns JP. Psychotic disorders in Prader-Willi syndrome. *Am J Med Genet A* 2004; **127A**: 238-243 [PMID: 15150773 DOI: 10.1002/ajmg.a.30004]

91 **Cassidy SB**, Schwartz S, Miller JL, Driscoll DJ. Prader-Willi syndrome. *Genet Med* 2012; **14**: 10-26 [PMID: 22237428 DOI: 10.1038/gim.0b013e31822bead0]

92 **Brøndum-Nielsen K**. The genetic basis for Prader-Willi syndrome: the importance of imprinted genes. *Acta Paediatr Suppl* 1997; **423**: 55-57 [PMID: 9401540 DOI: 10.1111/j.1651-2227.1997.tb18370.x]

93 **Cheon CK**. Genetics of Prader-Willi syndrome and Prader-Will-Like syndrome. *Ann Pediatr Endocrinol Metab* 2016; **21**: 126-135 [PMID: 27777904 DOI: 10.6065/apem.2016.21.3.126]

94 **Duker AL**, Ballif BC, Bawle EV, Person RE, Mahadevan S, Alliman S, Thompson R, Traylor R, Bejjani BA, Shaffer LG, Rosenfeld JA, Lamb AN, Sahoo T. Paternally inherited microdeletion at 15q11.2 confirms a significant role for the SNORD116 C/D box snoRNA cluster in Prader-Willi syndrome. *Eur J Hum Genet* 2010; **18**: 1196-1201 [PMID: 20588305 DOI: 10.1038/ejhg.2010.102]

95 **Wang SE**, Jiang YH. Potential of Epigenetic Therapy for Prader-Willi Syndrome. *Trends Pharmacol Sci* 2019; **40**: 605-608 [PMID: 31353046 DOI: 10.1016/j.tips.2019.07.002]

96 **Bieth E**, Eddiry S, Gaston V, Lorenzini F, Buffet A, Conte Auriol F, Molinas C, Cailley D, Rooryck C, Arveiler B, Cavaillé J, Salles JP, Tauber M. Highly restricted deletion of the SNORD116 region is implicated in Prader-Willi Syndrome. *Eur J Hum Genet* 2015; **23**: 252-255 [PMID: 24916642 DOI: 10.1038/ejhg.2014.103]

97 **Langouët M**, Glatt-Deeley HR, Chung MS, Dupont-Thibert CM, Mathieux E, Banda EC, Stoddard CE, Crandall L, Lalande M. Zinc finger protein 274 regulates imprinted expression of transcripts in Prader-Willi syndrome neurons. *Hum Mol Genet* 2018; **27**: 505-515 [PMID: 29228278 DOI: 10.1093/hmg/ddx420]

98 **Liu XS**, Wu H, Ji X, Stelzer Y, Wu X, Czauderna S, Shu J, Dadon D, Young RA, Jaenisch R. Editing DNA Methylation in the Mammalian Genome. *Cell* 2016; **167**: 233-247.e17 [PMID: 27662091 DOI: 10.1016/j.cell.2016.08.056]

99 **Cruvinel E**, Budinetz T, Germain N, Chamberlain S, Lalande M, Martins-Taylor K. Reactivation of maternal SNORD116 cluster *via* SETDB1

knockdown in Prader-Willi syndrome iPSCs. *Hum Mol Genet* 2014; **23**: 4674-4685 [PMID: 24760766 DOI: 10.1093/hmg/ddu187]

100 **Reiner D**, Seifert L, Deck C, Schüle R, Jung M, Stark H. Epigenetics meets GPCR: inhibition of histone H3 methyltransferase (G9a) and histamine H(3) receptor for Prader-Willi Syndrome. *Sci Rep* 2020; **10**: 13558 [PMID: 32782417 DOI: 10.1038/s41598-020-70523-y]

101 **Kim Y**, Lee HM, Xiong Y, Sciaky N, Hulbert SW, Cao X, Everitt JI, Jin J, Roth BL, Jiang YH. Targeting the histone methyltransferase G9a activates imprinted genes and improves survival of a mouse model of Prader-Willi syndrome. *Nat Med* 2017; **23**: 213-222 [PMID: 28024084 DOI: 10.1038/nm.4257]

102 **Kim Y**, Wang SE, Jiang YH. Epigenetic therapy of Prader-Willi syndrome. *Transl Res* 2019; **208**: 105-118 [PMID: 30904443 DOI: 10.1016/j.trsl.2019.02.012]

103 **Williams CA**, Driscoll DJ, Dagli AI. Clinical and genetic aspects of Angelman syndrome. *Genet Med* 2010; **12**: 385-395 [PMID: 20445456 DOI: 10.1097/GIM.0b013e3181def138]

104 **Huang HS**, Allen JA, Mabb AM, King IF, Miriyala J, Taylor-Blake B, Sciaky N, Dutton JW Jr, Lee HM, Chen X, Jin J, Bridges AS, Zylka MJ, Roth BL, Philpot BD. Topoisomerase inhibitors unsilence the dormant allele of Ube3a in neurons. *Nature* 2011; **481**: 185-189 [PMID: 22190039 DOI: 10.1038/nature10726]

105 **Lee HM**, Clark EP, Kuijer MB, Cushman M, Pommier Y, Philpot BD. Characterization and structure-activity relationships of indenoisoquinoline-derived topoisomerase I inhibitors in unsilencing the dormant Ube3a gene associated with Angelman syndrome. *Mol Autism* 2018; **9**: 45 [PMID: 30140420 DOI: 10.1186/s13229-018-0228-2]

106 **Tsagkaris C**, Papakosta V, Miranda AV, Zacharopoulou L, Danilchenko V, Matiashova L, Dhar A. Gene Therapy for Angelman Syndrome: Contemporary Approaches and Future Endeavors. *Curr Gene Ther* 2020; **19**: 359-366 [PMID: 31914913 DOI: 10.2174/1566523220666200107151025]

107 **Fink JJ**, Robinson TM, Germain ND, Sirois CL, Bolduc KA, Ward AJ, Rigo F, Chamberlain SJ, Levine ES. Disrupted neuronal maturation in Angelman syndrome-derived induced pluripotent stem cells. *Nat Commun* 2017; **8**: 15038 [PMID: 28436452 DOI: 10.1038/ncomms15038]

108 **van den Bos MAJ**, Geevasinga N, Higashihara M, Menon P, Vucic S. Pathophysiology and Diagnosis of ALS: Insights from Advances in Neurophysiological Techniques. *Int J Mol Sci* 2019; **20** [PMID: 31185581 DOI: 10.3390/ijms20112818]

109 **Nowicka N**, Juranek J, Juranek JK, Wojtkiewicz J. Risk Factors and Emerging Therapies in Amyotrophic Lateral Sclerosis. *Int J Mol Sci* 2019; **20** [PMID: 31141951 DOI: 10.3390/ijms20112616]

110 **Amado DA**, Davidson BL. Gene therapy for ALS: A review. *Mol Ther* 2021; **29**: 3345-3358 [PMID: 33839324 DOI: 10.1016/j.ymthe.2021.04.008]

111 **Longinetti E**, Fang F. Epidemiology of amyotrophic lateral sclerosis: an update of recent literature. *Curr Opin Neurol* 2019; **32**: 771-776 [PMID: 31361627 DOI: 10.1097/WCO.0000000000000730]

112 **Mejzini R**, Flynn LL, Pitout IL, Fletcher S, Wilton SD, Akkari PA. ALS Genetics, Mechanisms, and Therapeutics: Where Are We Now? *Front Neurosci* 2019; **13**: 1310 [PMID: 31866818 DOI: 10.3389/fnins.2019.01310]

113 **Trojsi F**, D'Alvano G, Bonavita S, Tedeschi G. Genetics and Sex in the Pathogenesis of Amyotrophic Lateral Sclerosis (ALS): Is There a Link? *Int J Mol Sci* 2020; **21** [PMID: 32455692 DOI: 10.3390/ijms21103647]

114 **Wang MD**, Little J, Gomes J, Cashman NR, Krewski D. Identification of risk factors associated with onset and progression of amyotrophic lateral sclerosis using systematic review and meta-analysis. *Neurotoxicology* 2017; **61**: 101-130 [PMID: 27377857 DOI: 10.1016/j.neuro.2016.06.015]

115 **Kim G**, Gautier O, Tassoni-Tsuchida E, Ma XR, Gitler AD. ALS Genetics: Gains, Losses, and Implications for Future Therapies. *Neuron* 2020; **108**: 822-842 [PMID: 32931756 DOI: 10.1016/j.neuron.2020.08.022]

116 **Meijboom KE**, Brown RH. Approaches to Gene Modulation Therapy for ALS. *Neurotherapeutics* 2022; **19**: 1159-1179 [PMID: 36068427 DOI: 10.1007/s13311-022-01285-w]

117 **Ediriweera GR**, Chen L, Yerbury JJ, Thurecht KJ, Vine KL. Non-Viral Vector-Mediated Gene Therapy for ALS: Challenges and Future Perspectives. *Mol Pharm* 2021; **18**: 2142-2160 [PMID: 34010004 DOI: 10.1021/acs.molpharmaceut.1c00297]

118 **Miller T**, Cudkowicz M, Shaw PJ, Andersen PM, Atassi N, Bucelli RC, Genge A, Glass J, Ladha S, Ludolph AL, Maragakis NJ, McDermott CJ, Pestronk A, Ravits J, Salachas F, Trudell R, Van Damme P, Zinman L, Bennett CF, Lane R, Sandrock A, Runz H, Graham D, Houshyar H, McCampbell A, Nestorov I, Chang I, McNeill M, Fanning L, Fradette S, Ferguson TA. Phase 1-2 Trial of Antisense Oligonucleotide Tofersen for SOD1 ALS. *N Engl J Med* 2020; **383**: 109-119 [PMID: 32640130 DOI: 10.1056/NEJMoa2003715]

119 **Kim A**, Lalonde K, Truesdell A, Gomes Welter P, Brocardo PS, Rosenstock TR, Gil-Mohapel J. New Avenues for the Treatment of Huntington's Disease. *Int J Mol Sci* 2021; **22** [PMID: 34445070 DOI: 10.3390/ijms22168363]

120 **Fields E**, Vaughan E, Tripu D, Lim I, Shrout K, Conway J, Salib N, Lee Y, Dhamsania A, Jacobsen M, Woo A, Xue H, Cao K. Gene targeting techniques for Huntington's disease. *Ageing Res Rev* 2021; **70**: 101385 [PMID: 34098113 DOI: 10.1016/j.arr.2021.101385]

121 **Tabrizi SJ**, Estevez-Fraga C, van Roon-Mom WMC, Flower MD, Scahill RI, Wild EJ, Muñoz-Sanjuan I, Sampaio C, Rosser AE, Leavitt BR. Potential disease-modifying therapies for Huntington's disease: lessons learned and future opportunities. *Lancet Neurol* 2022; **21**: 645-658 [PMID: 35716694 DOI: 10.1016/S1474-4422(22)00121-1]

122 **Pan L**, Feigin A. Huntington's Disease: New Frontiers in Therapeutics. *Curr Neurol Neurosci Rep* 2021; **21**: 10 [PMID: 33586075 DOI: 10.1007/s11910-021-01093-3]

123 **Feigin A**, Evans EE, Fisher TL, Leonard JE, Smith ES, Reader A, Mishra V, Manber R, Walters KA, Kowarski L, Oakes D, Siemers E, Kieburtz KD, Zauderer M; Huntington Study Group SIGNAL investigators. Pepinemab antibody blockade of SEMA4D in early Huntington's disease: a randomized, placebo-controlled, phase 2 trial. *Nat Med* 2022; **28**: 2183-2193 [PMID: 35941373 DOI: 10.1038/s41591-022-01919-8]

124 **Jurcau A**. Molecular Pathophysiological Mechanisms in Huntington's Disease. *Biomedicines* 2022; **10** [PMID: 35740453 DOI: 10.3390/biomedicines10061432]

125 **Duan W**, Urani E, Mattson MP. The potential of gene editing for Huntington's disease. *Trends Neurosci* 2023; **46**: 365-376 [PMID: 36907678 DOI: 10.1016/j.tins.2023.02.005]

126 **Tabrizi SJ**, Leavitt BR, Landwehrmeyer GB, Wild EJ, Saft C, Barker RA, Blair NF, Craufurd D, Priller J, Rickards H, Rosser A, Kordasiewicz HB, Czech C, Swayze EE, Norris DA, Baumann T, Gerlach I, Schobel SA, Paz E, Smith AV, Bennett CF, Lane RM; Phase 1–2a IONIS-HTTRx Study Site Teams. Targeting Huntingtin Expression in Patients with Huntington's Disease. *N Engl J Med* 2019; **380**: 2307-2316 [PMID: 31059641 DOI: 10.1056/NEJMoa1900907]

127 **Kumar D**, Hasan GM, Islam A, Hassan MI. Therapeutic targeting of Huntington's disease: Molecular and clinical approaches. *Biochem Biophys Res Commun* 2023; **655**: 18-24 [PMID: 36913762 DOI: 10.1016/j.bbrc.2023.02.075]

128 **Adil A**, Koritala T, Munakomi S, Singh AK. Neurofibromatosis Type 1. In: StatPearls. Treasure Island (FL): StatPearls Publishing, 2023.

Available from: http://www.ncbi.nlm.nih.gov/books/NBK459358/
129 **Evans DG**. Neurofibromatosis type 2 (NF2): a clinical and molecular review. *Orphanet J Rare Dis* 2009; **4**: 16 [PMID: 19545378 DOI: 10.1186/1750-1172-4-16]
130 **Tamura R**. Current Understanding of Neurofibromatosis Type 1, 2, and Schwannomatosis. *Int J Mol Sci* 2021; **22** [PMID: 34072574 DOI: 10.3390/ijms22115850]
131 **Williams VC**, Lucas J, Babcock MA, Gutmann DH, Korf B, Maria BL. Neurofibromatosis type 1 revisited. *Pediatrics* 2009; **123**: 124-133 [PMID: 19117870 DOI: 10.1542/peds.2007-3204]
132 **Kim Z**, Lee JH. Neurofibromatosis Symptom-Lacking B-Cell Lineage Acute Lymphoblastic Leukemia with Only an NF1 Gene Pathogenic Variant. *Diagnostics (Basel)* 2023; **13** [PMID: 37189588 DOI: 10.3390/diagnostics13081486]
133 **Parkin B**, Ouillette P, Wang Y, Liu Y, Wright W, Roulston D, Purkayastha A, Dressel A, Karp J, Bockenstedt P, Al-Zoubi A, Talpaz M, Kujawski L, Shedden K, Shakhan S, Li C, Erba H, Malek SN. NF1 inactivation in adult acute myelogenous leukemia. *Clin Cancer Res* 2010; **16**: 4135-4147 [PMID: 20505189 DOI: 10.1158/1078-0432.CCR-09-2639]
134 **Yap YS**, McPherson JR, Ong CK, Rozen SG, Teh BT, Lee AS, Callen DF. The NF1 gene revisited - from bench to bedside. *Oncotarget* 2014; **5**: 5873-5892 [PMID: 25026295 DOI: 10.18632/oncotarget.2194]
135 **Ly KI**, Blakeley JO. The Diagnosis and Management of Neurofibromatosis Type 1. *Med Clin North Am* 2019; **103**: 1035-1054 [PMID: 31582003 DOI: 10.1016/j.mcna.2019.07.004]
136 **Moutal A**, Yang X, Li W, Gilbraith KB, Luo S, Cai S, François-Moutal L, Chew LA, Yeon SK, Bellampalli SS, Qu C, Xie JY, Ibrahim MM, Khanna M, Park KD, Porreca F, Khanna R. CRISPR/Cas9 editing of Nf1 gene identifies CRMP2 as a therapeutic target in neurofibromatosis type 1-related pain that is reversed by (S)-Lacosamide. *Pain* 2017; **158**: 2301-2319 [PMID: 28809766 DOI: 10.1097/j.pain.0000000000001002]
137 **Chi XX**, Schmutzler BS, Brittain JM, Wang Y, Hingtgen CM, Nicol GD, Khanna R. Regulation of N-type voltage-gated calcium channels (Cav2.2) and transmitter release by collapsin response mediator protein-2 (CRMP-2) in sensory neurons. *J Cell Sci* 2009; **122**: 4351-4362 [PMID: 19903690 DOI: 10.1242/jcs.053280]
138 **Farrell CJ**, Plotkin SR. Genetic causes of brain tumors: neurofibromatosis, tuberous sclerosis, von Hippel-Lindau, and other syndromes. *Neurol Clin* 2007; **25**: 925-946, viii [PMID: 17964021 DOI: 10.1016/j.ncl.2007.07.008]
139 **Ater JL**, Zhou T, Holmes E, Mazewski CM, Booth TN, Freyer DR, Lazarus KH, Packer RJ, Prados M, Sposto R, Vezina G, Wisoff JH, Pollack IF. Randomized study of two chemotherapy regimens for treatment of low-grade glioma in young children: a report from the Children's Oncology Group. *J Clin Oncol* 2012; **30**: 2641-2647 [PMID: 22665535 DOI: 10.1200/JCO.2011.36.6054]
140 **Ullrich NJ**, Prabhu SP, Reddy AT, Fisher MJ, Packer R, Goldman S, Robison NJ, Gutmann DH, Viskochil DH, Allen JC, Korf B, Cantor A, Cutter G, Thomas C, Perentesis JP, Mizuno T, Vinks AA, Manley PE, Chi SN, Kieran MW. A phase II study of continuous oral mTOR inhibitor everolimus for recurrent, radiographic-progressive neurofibromatosis type 1-associated pediatric low-grade glioma: a Neurofibromatosis Clinical Trials Consortium study. *Neuro Oncol* 2020; **22**: 1527-1535 [PMID: 32236425 DOI: 10.1093/neuonc/noaa071]
141 **Kawachi Y**, Maruyama H, Ishitsuka Y, Fujisawa Y, Furuta J, Nakamura Y, Ichikawa E, Furumura M, Otsuka F. NF1 gene silencing induces upregulation of vascular endothelial growth factor expression in both Schwann and non-Schwann cells. *Exp Dermatol* 2013; **22**: 262-265 [PMID: 23528211 DOI: 10.1111/exd.12115]
142 **Randle SC**. Tuberous Sclerosis Complex: A Review. *Pediatr Ann* 2017; **46**: e166-e171 [PMID: 28414398 DOI: 10.3928/19382359-20170320-01]
143 **Zamora EA**, Aeddula NR. Tuberous Sclerosis. In: StatPearls. Treasure Island (FL): StatPearls Publishing, 2023. Available from: http://www.ncbi.nlm.nih.gov/books/NBK538492/
144 **De Waele L**, Lagae L, Mekahli D. Tuberous sclerosis complex: the past and the future. *Pediatr Nephrol* 2015; **30**: 1771-1780 [PMID: 25533384 DOI: 10.1007/s00467-014-3027-9]
145 **Northrup H**, Krueger DA; International Tuberous Sclerosis Complex Consensus Group. Tuberous sclerosis complex diagnostic criteria update: recommendations of the 2012 Iinternational Tuberous Sclerosis Complex Consensus Conference. *Pediatr Neurol* 2013; **49**: 243-254 [PMID: 24053982 DOI: 10.1016/j.pediatrneurol.2013.08.001]
146 **Miloloza A**, Rosner M, Nellist M, Halley D, Bernaschek G, Hengstschläger M. The TSC1 gene product, hamartin, negatively regulates cell proliferation. *Hum Mol Genet* 2000; **9**: 1721-1727 [PMID: 10915759 DOI: 10.1093/hmg/9.12.1721]
147 **Cheah PS**, Prabhakar S, Yellen D, Beauchamp RL, Zhang X, Kasamatsu S, Bronson RT, Thiele EA, Kwiatkowski DJ, Stemmer-Rachamimov A, György B, Ling KH, Kaneki M, Tannous BA, Ramesh V, Maguire CA, Breakefield XO. Gene therapy for tuberous sclerosis complex type 2 in a mouse model by delivery of AAV9 encoding a condensed form of tuberin. *Sci Adv* 2021; **7** [PMID: 33523984 DOI: 10.1126/sciadv.abb1703]
148 **Kohrman MH**. Emerging treatments in the management of tuberous sclerosis complex. *Pediatr Neurol* 2012; **46**: 267-275 [PMID: 22520346 DOI: 10.1016/j.pediatrneurol.2012.02.015]
149 **MacKeigan JP**, Krueger DA. Differentiating the mTOR inhibitors everolimus and sirolimus in the treatment of tuberous sclerosis complex. *Neuro Oncol* 2015; **17**: 1550-1559 [PMID: 26289591 DOI: 10.1093/neuonc/nov152]
150 **Bissler JJ**, McCormack FX, Young LR, Elwing JM, Chuck G, Leonard JM, Schmithorst VJ, Laor T, Brody AS, Bean J, Salisbury S, Franz DN. Sirolimus for angiomyolipoma in tuberous sclerosis complex or lymphangioleiomyomatosis. *N Engl J Med* 2008; **358**: 140-151 [PMID: 18184959 DOI: 10.1056/NEJMoa063564]
151 **Davies DM**, de Vries PJ, Johnson SR, McCartney DL, Cox JA, Serra AL, Watson PC, Howe CJ, Doyle T, Pointon K, Cross JJ, Tattersfield AE, Kingswood JC, Sampson JR. Sirolimus therapy for angiomyolipoma in tuberous sclerosis and sporadic lymphangioleiomyomatosis: a phase 2 trial. *Clin Cancer Res* 2011; **17**: 4071-4081 [PMID: 21525172 DOI: 10.1158/1078-0432.CCR-11-0445]
152 **Verhave J**, Boucher A, Dandavino R, Collette S, Senécal L, Hebert MJ, Girardin C, Cardinal H. The incidence, management, and evolution of rapamycin-related side effects in kidney transplant recipients. *Clin Transplant* 2014; **28**: 616-622 [PMID: 24654608 DOI: 10.1111/ctr.12361]
153 **Fidan K**, Kandur Y, Sozen H, Gonul II, Dalgic A, Söylemezoğlu O. How often do we face side effects of sirolimus in pediatric renal transplantation? *Transplant Proc* 2013; **45**: 185-189 [PMID: 23375296 DOI: 10.1016/j.transproceed.2012.08.005]
154 **Śmiałek D**, Jóźwiak S, Kotulska K. Safety of Sirolimus in Patients with Tuberous Sclerosis Complex under Two Years of Age-A Bicenter Retrospective Study. *J Clin Med* 2023; **12** [PMID: 36615165 DOI: 10.3390/jcm12010365]

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Personalized clinical managements through exploring circulating neural cells and electroencephalography

Parvin Mehdipour, Nima Fathi, Masoud Nosratabadi

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Parvin Mehdipour, Department of Medical Genetics, Tehran University of Medical Sciences, Tehran 14176-1315, Iran

Nima Fathi, Neuro-Science, Paarand Specialized Center for Human Enhancement, Tehran 157699304, Iran

Masoud Nosratabadi, Department of Research, Paarand Specialized Center for Human Enhancement, Tehran 157699304, Iran

Corresponding author: Parvin Mehdipour, PhD, Full Professor, Department of Medical Genetics, Tehran University of Medical Sciences, Pour Sina Ave, Tehran 14176-1315, Iran. mehdipor@tums.ac.ir

Abstract

BACKGROUND

Since an initial diagnosis of Alzheimer disease (AD) in 1907, early detection, was unavailable through 116 years. Up-regulation of V-Ets erythroblastosis virus E26 oncogene homolog 2 (Ets2) is capable to enhance neuronal susceptibility and degeneration. Protein expression (PE) of Ets2 has functional impact on AD and Down's syndrome, with diverse intensity. PE of Ets2 has an influential pathogenic impact on AD. Clinical aspects of neurological disorders directly interact with psychological maladies. However, deterioration requires an early management including programmed based protection.

AIM

To include cell biology in neuro-genetics; personalized, prognostics, predictive, preventive, predisposing (5xP) platform, accompanied by stratifying brain channels behavior pre- and post-intervention by light music in the AD-patients.

METHODS

Include exploration of PE assay and electroencephalography of brain channels. The processes are applied according to: (1) Triangle style, by application of cellular network; and (2) PE assay of Ets2 in the peripheral blood of the patients with AD, by Manual single cell based analysis, and Flow-cytometry. (1) Applying the Genetic counselling and pedigree analysis; (2) considering the psychological status of the referral cases; (3) considering the macro-and/or micro-environmental factors; (4) performing the required Genetics' analysis; and (5) applying the required complementary test(s).

RESULTS

PE of Ets2 has pathogenic role in AD. PE unmasked the nature of heterogeneity/diversity/course of evolution by exploring Ets2, D1853N polymorphism in Ataxia Telangiectasia mutated gene (ATM), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and course of evolution at the single cell level of the brain. Ets2 revealed different cellular behavior in the blood and suggested the strategy as 'Gene Product-Based Therapy' and the personalized managements for the patients. PE reflected weak expression of ATM, mosaic pattern of Ets2; remarkable expression of VEGF and EGF by highlighting an early detective platform, considering circulating neural cells (CNCs) and the required molecular investigation, for the target individual(s) predisposed to AD or other neural disease including brain neoplasia. Brain channels-cooperation with diverse/interactive-ratios lead to strategic balancing for improving the life-quality in AD.

CONCLUSION

We highlighted application of the single CNCs and correlated Ratio based between Brain channels by providing the 5xP personalized clinical management model for an early detection and therapy of the patients with AD and their targeted/predisposed relatives. Novel-evolutionary/hypothetic/heterogenic-results in brain-channels offer personalizd/constructive markers with unlimited cooperation in health and disease.

Key Words: Alzheimer; Protein expression; Brain channels; Predictive/early detection

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Core Tip: The provided outcomes emphasize on the personalized/classified/functional/single cell based/complementary insights, and systematic strategy in Neuro-Science. The successful bridging approach between Neuro-Science and Medicine requires: (1) The combination of the molecular and functional insights at single cell level; (2) by emphasizing on the course of evolution; and (3) to expedite towards unmasking the functional modifications in the blood stream of the patients with neurological disorders including Alzheimer disease (AD). However, exploration of the circulating neural cells accelerate to unmask the course of evolution by providing the personalized and translatable model to the target based therapy. Let's improve the life quality of the patients with neurological disorders including AD with simply the light music which corresponds with 40 Hz in gamma sensory stimulation therapy. It is essential to differentiate between neurological- and neuro-psychiatric diseases. Surprisingly, we offer an early detection of the stem cells, including the neural CD133 at fetal period, *i.e.,* as early as 8-9 wk, or later through the circulating fetal cells in the maternal blood stream.

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INTRODUCTION

Present introduction provides the multi-directional insights towards the Alzheimer disease (AD), including the function of involved protein expression (PE) at single cell level; and electroencephalography (EEG), to observe the role of light music (LM) on the brain channels, and the interaction between different brain channels. It was also aimed to provide the personalized periodic chart for the predisposed individual and/or patients affected with AD. In addition, an initial description of the histopathology of AD has been provided by Alois Alzheimer[1] in 1907, which is categorized by brain atrophy, an extracellular, cumulated of Aβ peptide (amyloid plaques), loss of neuron and synapse, composition of tau protein, as neurofibrillary ingredient[1]. Regulation of the Human Presenilin-1 Gene by V-Ets erythroblastosis virus E26 oncogene homolog 2 (Ets2) Transcription Factors and p53 play key role in AD-pathogenesis[2]. The decisive role of DNA by controlling 90% of the expression of presenilin 1 gene located in chromosome 21 (-35 to +6) is highlighted. This region harbors an Ets transcription factor-binding motif, and a 2-base pair alteration within the sequence of (GGAA to TTAA) of the Ets2 territory leading to the 90% transcriptional reduction. It was also reported that Ets1/2 transcription factors activate PS1 transcription.

The pathological diagnosis in AD, and accumulation of amyloid, occur almost decades before an initial sign of clinical symptoms[3]. Besides, functional aspect of brain network could be unmasked by magnetoencephalography. Most importantly, application of the non-invasive strategy, as an early detection of the symptom(s) of neuropathology at pre-developmental period of the AD is essential.

Initially, the micro- and macro-environmental factors, including nutrition play, partly, the influential roles in initiation and progression of AD. The most challenging panel in this topic is the late diagnosis and the risky procedures through the exploration of AD and therapy. In addition, EEG is the non-invasive and trustable method to detect synaptic dysfunction and the course of the disease. However, performance of EEG is helpful, but very late.

In addition, the combined strategy to classify the EEG-data, by applying an early functional assay of the target protein(s), at single cell level, and as the personalized analytical procedures in AD is, currently, our ongoing process.

Brain function could be, routinely, examined by EEG in AD with significant resolution[4]. EEG is revealed to be: (1) Non-invasive; (2) unmasks the alphabetic informative codes of the neural territory; (3) recording many required targets; (4) unmasking the health conditions related to the patients' age; and (5) deals with the pharmacological modeling[5,6]. Actually, EEG is capable to unmask the basic prognostic mechanisms in AD. Furthermore, it is proposed that the progression in AD is supposed to be associated to the "functional disconnection"[7].

In the resting phase, the increased and decreased functional connection is indicative of activity for the prefrontal and posterior regions through the alpha band[8,9]. Besides, by considering the decreased function in EEG exploration, its consequences on the connectivity is remarkable which depends on the individuals and technology.

Regarding the genetic test, the Ets2 up-regulation may lead to an increased neuronal degeneration, apoptosis and susceptibility[10]. The *Ets2* gene is involved in two different group of patients, affected at totally diverse age of onset including AD and Down's syndrome (DS)[11].

The primary predisposing/predictive/early detective strategy is the pedigree-based analysis of the proband affected with nervous system/brain disorder(s) including AD. Such approach will cover the candidate relatives of the proband(s) for the screening program. This strategy will unmask: (1) The predisposing factor through the pedigree; and (2) the target and candidate relatives for an early and non-invasive screening.

Circulating neural cells (CNCs) offer: (1) The non-invasive screening; (2) at single cell level; and (3) unmasking the heterogeneity/diversity and evolution.

Ethics play fundamental and supportive role in Genetics and Psychiatry. It facilitates, care and guarantee the benefit of patients in different stages including sampling, research, and clinical managements. Through such platforms, the chain of events including predisposition, prognosis, prediction, prevention (as 4xP aim), diagnosis and personalized therapy are required to be considered. In cell biology, the single cell level play the key role in unmasking the brain's behavior and have complimentary role and functional impact on the neurological system.

The personalized, prognostics, predictive, preventive, predisposing (5xP) strategy would be achievable through the systematic exploration with an early-detection and personalized therapeutic management. Besides, the pedigree-based analysis provides the predictive stage for the relatives of proband, affected with AD at any age.

Personalized screening, at single cell level, with adequate cell analysis, as early as possible, is scientifically trustable. Therefore, it is an urgent aim for both Scientific and Medical platforms to consider an early detection of the neural disorders.

Brain is a sophisticated and transmittable organ. The pathology of the brain is classified and available[3]. By considering Genetics and cell biology, the specific factors in the neural cells play key role in the neural territory. As a result, the manner of current systems have the influential and functional roles on the specific neurological mechanisms at single cell level. Therefore, to unmask the alterations, it requires the most comprehensive exploration at somatic- and genomics level[11].

Brain harbors the multi-channels, which is characterized with the miscellaneous and essential proteins.

Conclusively, neural cells are derived from the pool of homogeneous progenitor cells. Furthermore, environmental factors, affect the genetic variations and predisposing factors. Concerning the required techniques and the essential therapeutic aspects of the neurological disorders, the informative panel including the histo-pathologic, PE at single cell level and the molecular techniques are required.

As the routine manner, the most puzzling information achieved at the global level, which does not provide the essential Information on the diversity and heterogeneity. Furthermore, the single cell based analysis of PE, is capable to unmask the architecture of the expression and co-expression at the final cellular procedure and production.

The routine and global molecular based analysis are, rapidly, used in the neuroscience research and diagnosis, but are not informative to unmask the heterogeneity and diversity of the functional single cell based which has key role on the occurrence of evolution.

As the matter of fact, the roots of different diseases including cancer and non-cancerous are either related to their ancestral lines and sides through the pedigrees, or the rules and natural events at embryonic, and/or fetal, adulthood and elderly durations (Figure 1). However, micro- and macro-environmental factors play the influential role to initiate and developing disorders. Finally, the complicated periodic chart is required to be considered in the management of health and diseases for the index case and their targeted relatives through their pedigrees.

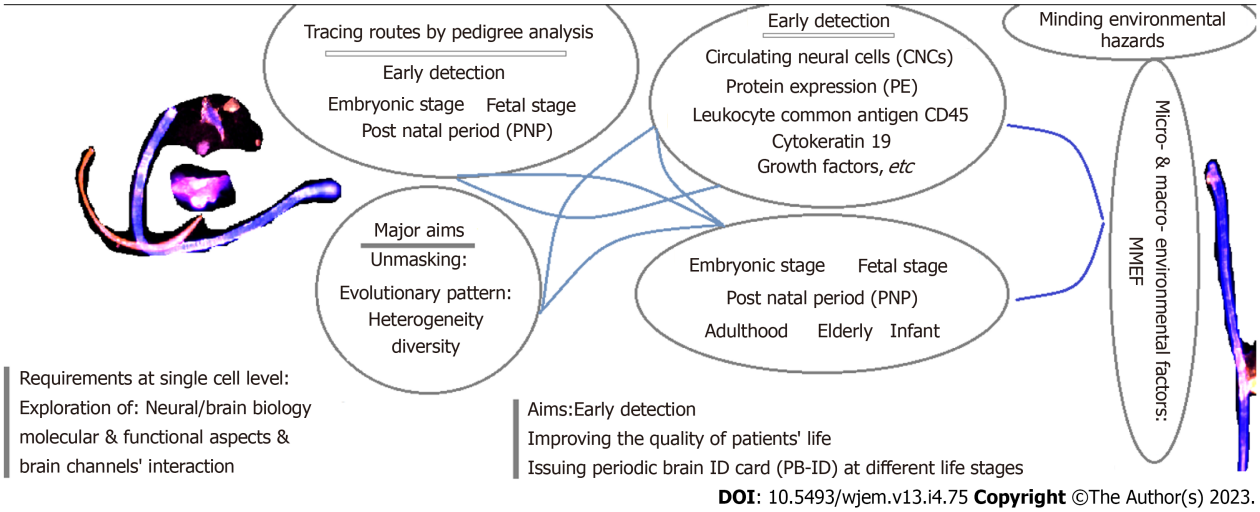
Cancer and Alzheimer are the historical diseases and have, their roots, hidden at the single cells and brain territory.

The physicians and scientists need to take their valuable action as the experienced archeologists, to trace the roots of both diseases, one by one at single cell level and not at global dimension.

Figure 1 presents the flow-chart which may assist the medical team, including clinicians, pharmacologists, nurses, and scientists to achieve the required complementary data, as early as possible. The prompt therapy requires an early diagnosis. At a glance, the matter of fact includes the occurrence and diagnosis of diversity, heterogeneity and evolution. Such a triangle, will direct the medical, pharmaceutics and scientific teams to: (1) The translatable platform; and (2) to plan the personalized clinical management including an early-therapy (Figure 1).

MATERIALS AND METHODS

Ten patients, including six females and four males, affected with AD; and ten healthy individuals are participated in our previous study[11]. The lymphocytes were cultured in RPMI media (Sigma Aldrich, St Louis, MO, United States) for 35



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Figure 1 The required managements for early detection and therapy: A Journey from embryonic and fetal periods to elderly.

min at 37 °C. Then the cells were treated with the hypertonic solution, and were fixed. The lymphocytes were stained with the polyclonal Ets2 antibody (Avivasysbio, CA, United States), washed by Phosphate buffer solution, and then stained with secondary antibody (FITC-conjugated goat-anti-rabbit). Flow-cytometry (FC) assay was performed by BD FACS Calibur FC (BD FACSCALIBUR™ FC-System, United States) and the results were analyzed by Flowjo-7.6. software.

Data was analyzed by SPSS 18 (SPSS Inc, IL, United States) The Spearman and Pearson Correlation Coefficients were also performed. The, *P* value less than 0.05 was considered as significant[11].

The single cell based assay is an essential channel to unmask heterogeneity, diversity, and evolution. Besides, the high cell enumeration is required. There are apparatus; capable to automate, cell analysis, but very few cells is enumerated. Besides, through the manual analysis, broad exploration of multi thousand single cells are analyzable to unmask the new cellular function and their interaction.

PE has been assayed by

Manual single cell based analysis by immunofluorescence (IF), also based on high enumeration, with different magnification, which provides the classified intensity of the PE. However, FC is totally machinery based and non- classified cellular screening according to the degree of intensity of the whole cell population with manual IF, and FC.

Visions and visualization: PE assay and EEG

The applied processes are performed according to the triangle style, by highlighting the power of cellular network, which reflects the association between the PE of Ets2 is also performed in the peripheral blood of the patients with AD, according to the standard flow cytometry technique[11]. The PE assay was capable to unveil the nature of heterogeneity/diversity/course of evolution by exploring D1853N polymorphism in ATM gene, and PE assay of vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) at the single cell level of the brain and through the course of evolution[12,13].

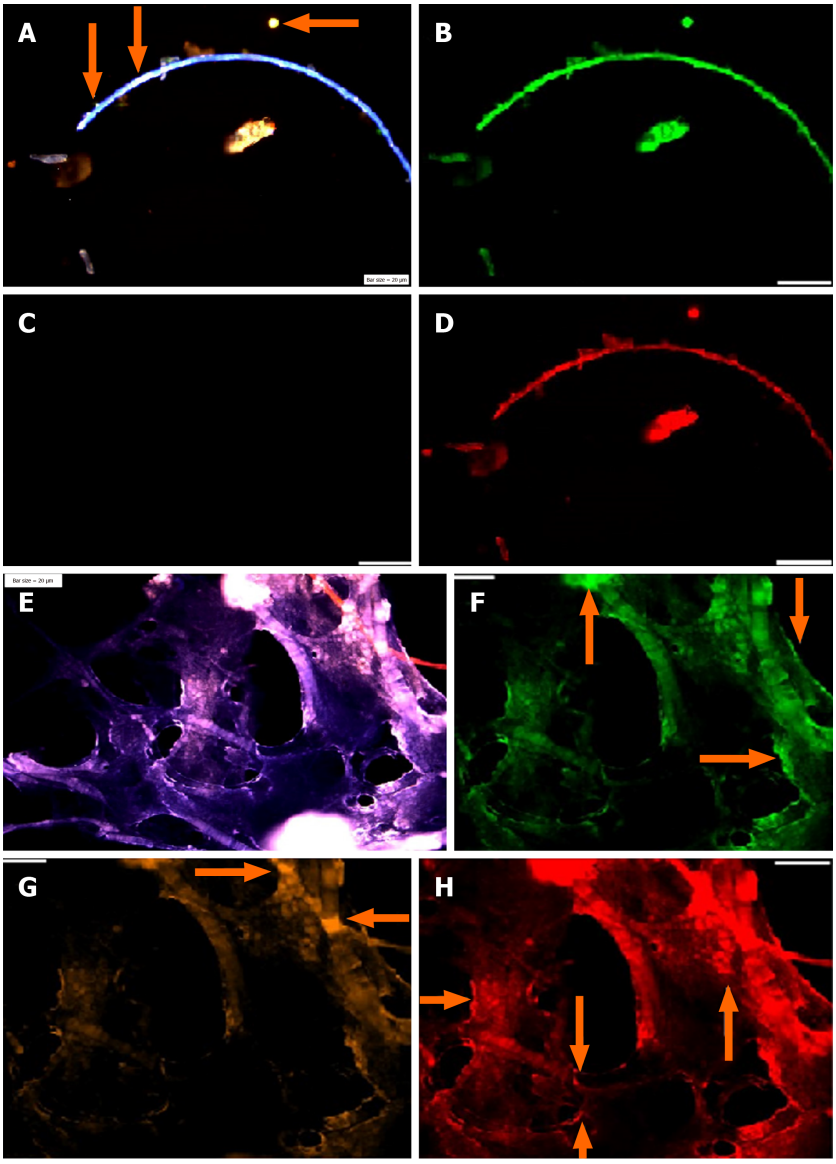
However, there are challenges regarding the CNCs in neurological disorders[14]. Furthermore, an innovative and complementary insight is required for an intensive exploring of the brain channels as well. Buzsáki *et al*[14] have focused on "The origin of extracellular fields and currents-EEG, ECoG, LFP and spikes"[14]. Briefly, the challenges and achieved data in circulating tumor cells, and EEG highlights the involvement of two diverse territories and the related machinery in AD[14,15].

By considering the presence of family history of AD in the related pedigree; CNCs is offered as an applicable biomarker based test: (1) At any age, by only 2 mL of the pregnant maternal peripheral blood to screen the status of target embryo and/or fetus for Ets2 gene and the CNCs; and (2) screening the same test by buccal mucosa or buccal smear of the born child at different stages of his or her life.

RESULTS

PE assay

Analysis of PE was performed in the embryonic and chorionic villus samples; and patients affected with AD. PE is assayed by IF in the peripheral blood samples and 1000-3000 cells were analyzed at single cell level, by the manual exploration. An aborted embryonic sample at the early gestational week has been explored to assay the mode of PE, including neural marker (NE), neural stem cell (CD133) and VEGF (Figure 2A-C). PE at early stage of embryonic period revealed to be high for NE (Figure 2A) and VEGF (Figure 2C). But, the Neural stem cell marker (CD133) is characterized with an absolutely lack of PE, which reflects no sign of stem cell function at early stage of embryo in this aborted sample

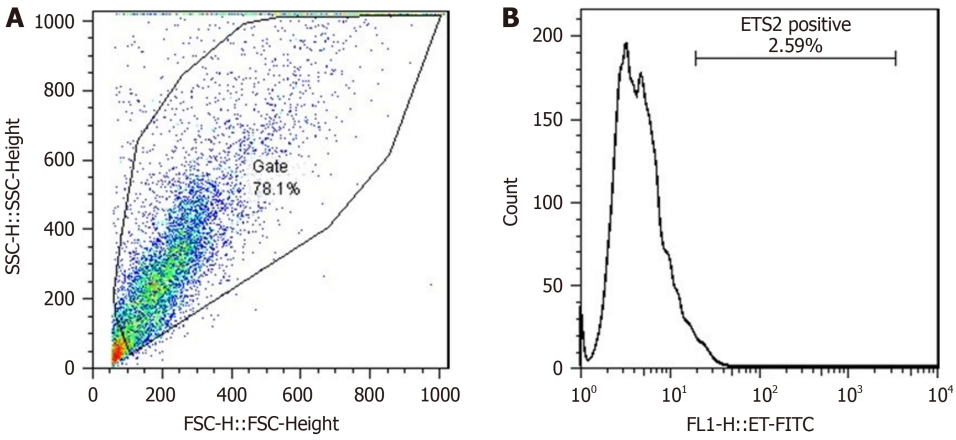


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Figure 2 Circulating neural cells in embryonic and chorionic villus samples. A: Circulated neural cells (CNCs) conjugated with Dapi/Ne/CD133/vascular endothelial growth (VEG); B: Same cells conjugated with fluorescein isothiocyanate (FITC) (Ne) reflecting high protein expression in the limited cells; C: Same cells conjugated with Rpe (CD133), totally lacks expression in stem cells; D: Same cells conjugated with Pe-Cy5, reflecting high protein expression, accompanied by few cells with low expression; E-H: Reflect protein expression in chorionic villus sample including; E: CNCs conjugated with Dapi/Ne/CD133/VEG; F: Same cells conjugated with FITC (Ne) reflecting high protein expression; G: Same cells conjugated with Rpe (CD133), lack of expression is observable, accompanied by few cells with positive CD133; H: Same cells conjugated with Cy5 (VEGF), reflecting high protein expression and remarkable angiogenesis. Bars = 20 μm.

(Figure 2A-D).

Regarding the chorionic villus, sample, the behavior of PE seems to be diverse, the highest PE is traced for VEGF with high angiogenesis in the whole sample (Figure 2E-H); mosaic pattern of PE for the NE is more remarkable (Figure 2B) than VEGF, the stem cell CD133, reveals a notable, low expression, accompanied by few cells with high expression (Figure 2E-H) which reflect the initiative step for activation of CD133 stem cell, and significantly differs from the embryonic sample, lacking any expression of CD133. This is the course of evolution, which could be influenced by micro- and macro environmental factor during the fetal growth. Such behavior could occur for any other involved marker(s) for cancer initiation and/or progression. By projecting the impact of early detection, the initial target to unmask any sign of functional alteration related to the neurological disorders including AD by circulating fetal cells (CFCs), is available. In this regard, we offer an extremely early detection of neural stem cell (CD133), Ets2, and any related key proteins, involved in the initiation and progression of neurological disorders including AD at 8-9 gestational week (Figure 2E-H). Furthermore, screening the evolutionary course of the molecular and functional alteration(s) is possible by the follow-up strategy and only 2-3 mL maternal peripheral blood as often as, is essential. In addition, we provide an early detective platform including the stem cells (CD133) at fetal period, *i.e.*, as early as 8-9 wk through the CFCs/chorionic villus in the maternal blood stream. Besides the key proteins including cyclins B, D, E, EGFs and VEGF could be also analysed. The benefit of such an extremely early screening is to deliberate the preliminary and preventive strategy including micro- and



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Figure 3 Flow-cytometry results of erythroblastosis virus E26 oncogene homolog 2 protein expression in a patient affected with Alzheimer disease. A: R1: 78.1 % (7812/10000 cells); B: Erythroblastosis virus E26 oncogene homolog 2: 2.59 % (202/7812 cells). Ets2: Erythroblastosis virus E26 oncogene homolog 2; SSC: Side scatter reflects the internal complexity. Adapted from Reference[11].

macro-environmental factors, counting nutrition. Besides, planning the essential and non-invasive screening, such as EEG at the right time through the offspring's life, under the guide and care of physician could be planned to detect any sign of AD manifestation(s) for applying any essential 5xP aims.

Besides the manual detection by immunofluorescence, automated analysis of PE by FC, is performed for Ets2 protein (Figure 3).

Three proteins including ATM, Ets2 and VEGF are reflective of: (1) Weak expression of ATM as a tumor suppressor gene; (2) mosaic pattern of PE for Ets2; and (3) remarkable expression of VEGF which is indicative of angiogenesis in the neural cells (Figure 4).

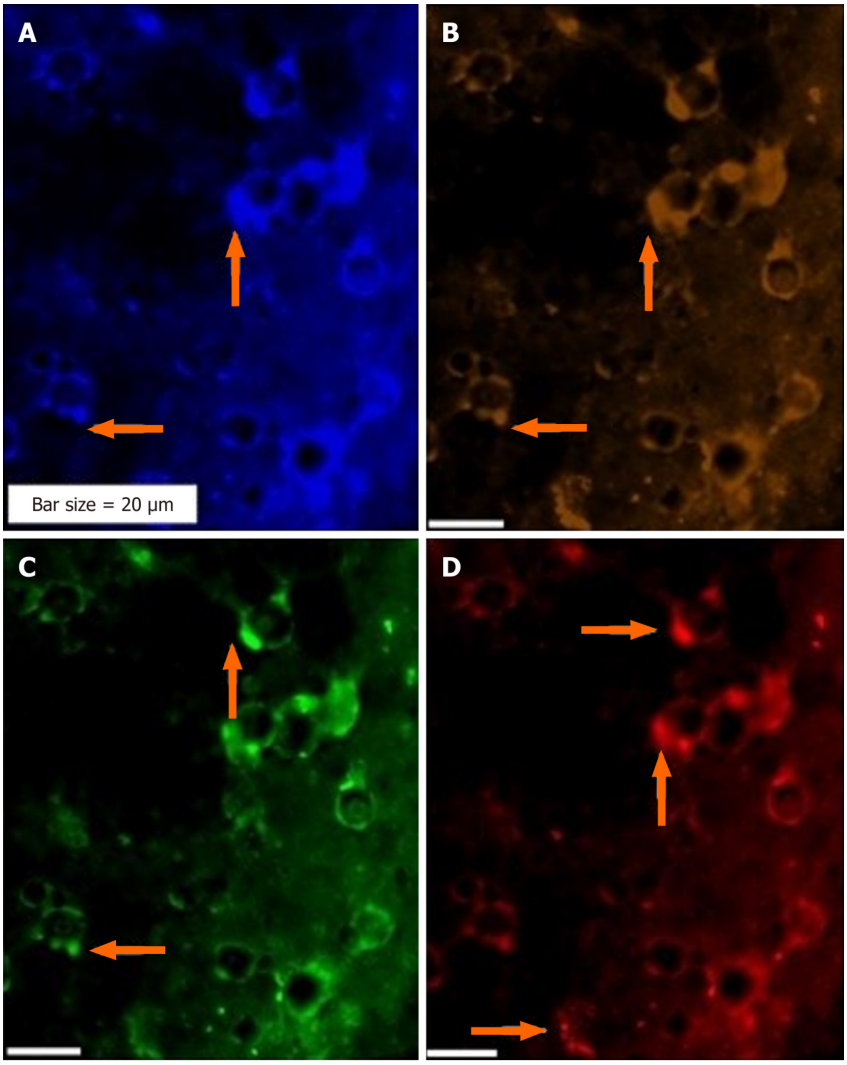
The CNCs were explored by IF method for combination of NE, neural stem cell and angiogenesis (NE/CD133/VEGF (Figures 5 and 6); and for Ne/CD133 (Figure 7). The PE of NE/VEGF/Ets2 is also provided (Figure 8). The migrated neural cells with high or low expression are shown with arrows. The micro-vesicle and the vascular section harboring the migrated neural cells are detectable. The CNCs were explored by IF method for combination of NE and neural stem cell (NE/CD133) (Figure 7). The PE of NE/VEGF/Ets2 is also provided (Figure 8). Lower expression of this triangle (NE/VEGF/Ets2) is a reliable predictive/early and non-invasive platform for exploring the status of AD at any stage of life. The CNCs' ratio, between pre- and post- intervention were analyzed based on the PE-screening of cytokeratin 19 (CK19), and leukocyte common antigen (CD45) and EGF. The cells conjugated with VEGF (Rpe), having lower expression (Figure 8), the CNCs' ratio, between pre- and post- intervention were analyzed based on the PE-screening of CK19, and leukocyte common antigen (CD45) and EGF. The personalized insight is considered/and the ratio value is estimated for the cases. The Ratios are characterized as < 1 and > 1 between different channels within the horizontal and vertical axes. Finally, the diverse patterns were classified according to the distribution including clusters or sporadically forms. The CNCs' ratio, between pre- and post- intervention were analyzed based on the PE- screening of CK19, leukocyte common antigen (CD45) and EGF (Figure 9). The confirmation of CNCs was based on the mode of positive $CK19^+$ and negative CD45. The personalized insight is considered. The ratio value is estimated in cases. The Ratios are characterized as < 1 and > 1 between different channels within the horizontal and vertical axes. Finally, the diverse patterns were classified according to the distribution including clusters or sporadically forms.

Conclusively, by aiming an early clinical management, including diagnosis and therapy, the application of an available multi-panels based on: (1) Non-Invasive; (2) repeatable; (3) cost benefit; and (4) safe CNCs sample, in only 2 mL peripheral blood or buccal smear, for an early detection of the target proteins expression in the brain cells could be considered. Let's Revise Alzheimer disease, based on single cell based vocabulary (Figure 10).

Applicability at a glance

The combination of neurology/genetics/cell biology/psychology/ethics is required for the research and clinical management for the neurological disorders. Moreover, the following items harmonize the 5xP by: (1) Applying the Genetic counselling by documenting the patients' pedigree, including the family history of the related diseases, up to 3-4 or more generations; (2) organization of the accustomed plan according to the psychological status of the referral cases; (3) considering the macro-and/or micro-environmental factors in the patients' pedigrees; (4) performing the required Genetics' analysis according to the information, based on the pedigree study; (5) focusing on the apparently normal relatives, having the family history of the relatives affected with the diseases through generations in the pedigree; (6) applying the required complementary test(s); and (7) application of the predictive and early detective strategy for the target suspicious patients with AD and their target relatives.

Genetics/cell biology organize and play the most fundamental roles, by considering a triangle model to unmask the heterogeneity/diversity/evolution. Through such manner, the personalized diagnosis and therapy could be developed. AD as a major aim is selected to explore Ets2. This gene has been explored in two different group of patients affected with neuro-degenerative disorder including AD and DS[11]. Ets2 with its pathogenic role, revealed different cellular behavior



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Figure 4 Protein expression of Ataxia Telangiectasia mutated gene, erythroblastosis virus E26 oncogene homolog 2, and vascular endothelial growth factor in the circulated neural cells from the neural system to the blood stream in a patient with Alzheimer disease. A: Circulated neural cells (CNCs) with dapi; B: CNCs conjugated with Ataxia Telangiectasia mutated (Rpe), reflecting lack or very low expression; C: The same cells conjugated with Ets2 (Fluorescein isothiocyanate) with a mosaic pattern of expression; D: Same cells conjugated with vascular endothelial growth factor (Rpe), reflecting mosaic angiogenesis.

in two different diseases. The results have suggested the strategy as 'Gene Product-Based Therapy' and the personalized managements for both diseases.

DISCUSSION

The information of patients' and controls are provided in Tables 1 and 2. Total cells of 10000 in AD patients were analyzed by FC, but without providing any heterogeneity. The FC-results of Ets2, is provided (Figure 3). PE assay unmasked the nature of heterogeneity/diversity/course of evolution by exploring Ets2, D1853N polymorphism in ATM gene, VEGF, EGF and course of evolution at the single cell level of the brain. The arrangement of Genetics/Cell biology/Psychology/Ethics is essential quadrat-radial format for programming the research and clinical management. The required steps in the Genetics and cell biological programs include: (1) Genetic counselling/pedigree documentation; (2) considering the macro-and/or micro-environmental factors; (3) performing the required Genetics' analysis, based on the pedigree-information; (4) considering the target proband's relatives for the required screening; and (5) applying the required Genetic test(s). Besides, harmonizing the 5xP insights are the essential target in the clinics.

Complementary and comparative insights between different panels of PE assay is the key aim in the patients with AD. PE of Ets2 by IF at analyzable single cell level of the AD patients are provided (Figures 4 and 9). However, PE is a challenging assay and the cells with high magnification are required to: (1) Reflect the clear panorama of the PE; and (2) most importantly, to echo the cellular heterogeneity clearly. The PE of Ets2 is also assayed for ten control individuals (Table 2). Two different single cell- based platforms are indicative of diverse conclusions. In fact, the most diagnostic and

Table 1 Erythroblastosis virus E26 oncogene homolog 2 protein expressions status and clinical features of Alzheimer's disease patients

Case No	Gender	Age, yr	Clinical feature	Family History of AD (years old)	Ets2 protein expression (%)	Total cells evaluated by flow-cytometry
1	F	69	ML	-	146 (3.47)	4207
2	F	57	ML, tremble (hands)	Cousin of paternal father (65)	155 (2.13)	7276
3	F	92	ML, MD	-	121 (1.11)	10900
4	M	76	ML	Half brother (70)	607 (15.8)	3841
5	M	79	ML	-	810 (11)	7363
6	M	67	ML (severe)	-	166 (2.43)	6831
7	M	42	ML (severe)	-	1100 (6.75)	16296
8	F	54	ML	-	228 (3)	7600
9	F	81	ML, HC, BP	Maternal aunt (95)	98 (5.12)	1914
10	F	79	ML	-	541 (18.9)	2862

M: Male; F: Female; ML: Memorial loss; MD: Movement disorder; HC: Heart complication, BP: High blood pressure; AD: Alzheimer disease; Ets2: Erythroblastosis virus E26 oncogene homolog 2; Total cells analyzed in 10 patients: 69090[11].

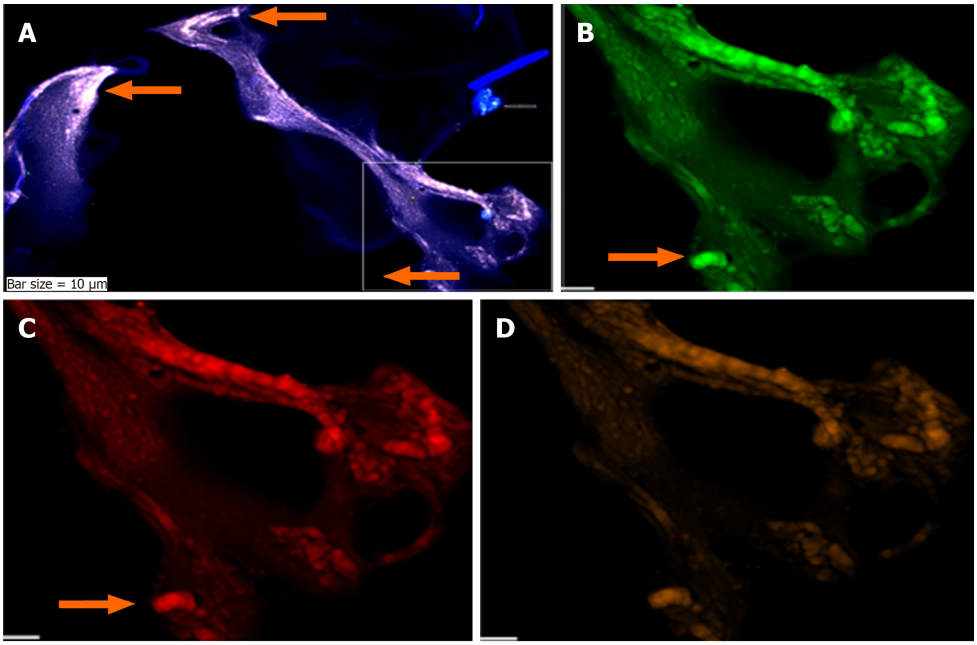
Table 2 Erythroblastosis virus E26 oncogene homolog 2 protein expressions status in healthy individuals as control for the patients with Alzheimer disease

ID	Age, yr	Ets2 protein expression (%)	Total cells analyzed
C-1	65	2 (0.066)	3025
C-2	56	10 (0.19)	5214
C-3	79	0 (0)	1546
C-4	74	3 (0.065)	4587
C-5	79	6 (0.48)	1232
C-6	62	3 (0.38)	783
C-7	42	0 (0)	923
C-8	54	4 (0.10)	9823
C-9	81	7 (004)	874
C-10	79	3 (0.19)	1542
Total		38 (0.12)	29549

The cells were analysed by flow cytometry: All controls (C) had no family history of Alzheimer disease. C: Control; Ets2: Erythroblastosis virus E26 oncogene homolog 2.

visibility of cellular diversity is achievable by IF through which the territory of the migrated cells from the brain domain is detectable (Figures 3 and 8). The provided figure by IF, clearly, reflect heterogeneity at single cell level.

An extra copy of Ets2 gene in the DNA of brain cells is reported by quantitative densitometry[16] and the provided guidlines[17,18]. Based on this valuable finding, the possible correlation of an extra chromosome 21 in DS as the cause of AD was also reported. In this regard, the expression of Ets2 was also high in DS by: (1) A segmental trisomy model for transcriptome during postnatal development of DS in a mouse model[19]; (2) leading to induction of neural apoptosis [20]; and (3) high-expression of Ets2 led to amplification of the APP gene expression and raising β-amyloid proteins, with consequential brain anomaly in AD and DS[21]. The Most interesting report was related to the beta amyloid gene duplication in both Alzheimer's disease and the case of DS with normal karyotype[21]. It has been, also, emphasized that through the mechanism of an imbalance process for gene dosage, the mental disorder could be evolved through the molecular/cellular machinery in DS[22]. Conclusively, the PE of Ets2, as a remarkable pathogenic factor, has the fundamental role at single cell level of two different disease including AD and DS.



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Figure 5 Protein expression of neural marker, CD133, and vascular endothelial growth factor in the circulated neural cells from the neural system to the blood stream in a patient with Alzheimer disease. A: Merged of dapi/fluorescein isothiocyanate/CD133/vascular endothelial growth factor (VEGF); B: Neural cells, conjugated with FITC with low expression, accompanied by the cells with high expression; C: Neural cells conjugated with Pe-cy5 also with low expression, accompanied by the cells with high expression; D: Cells conjugated with R-phycoerythrin and VEGF, majority of cells reflecting low expression, accompanied with a minor cells with lack of expression.

It is reported that application of "Sensory-Evoked 40-Hz Gamma" facilitates and improves sleeping process and the quality of life in the patients affected with Alzheimer's Disease[23]. Similarly, the light music has also the same impact on the individuals affected with AD as well.

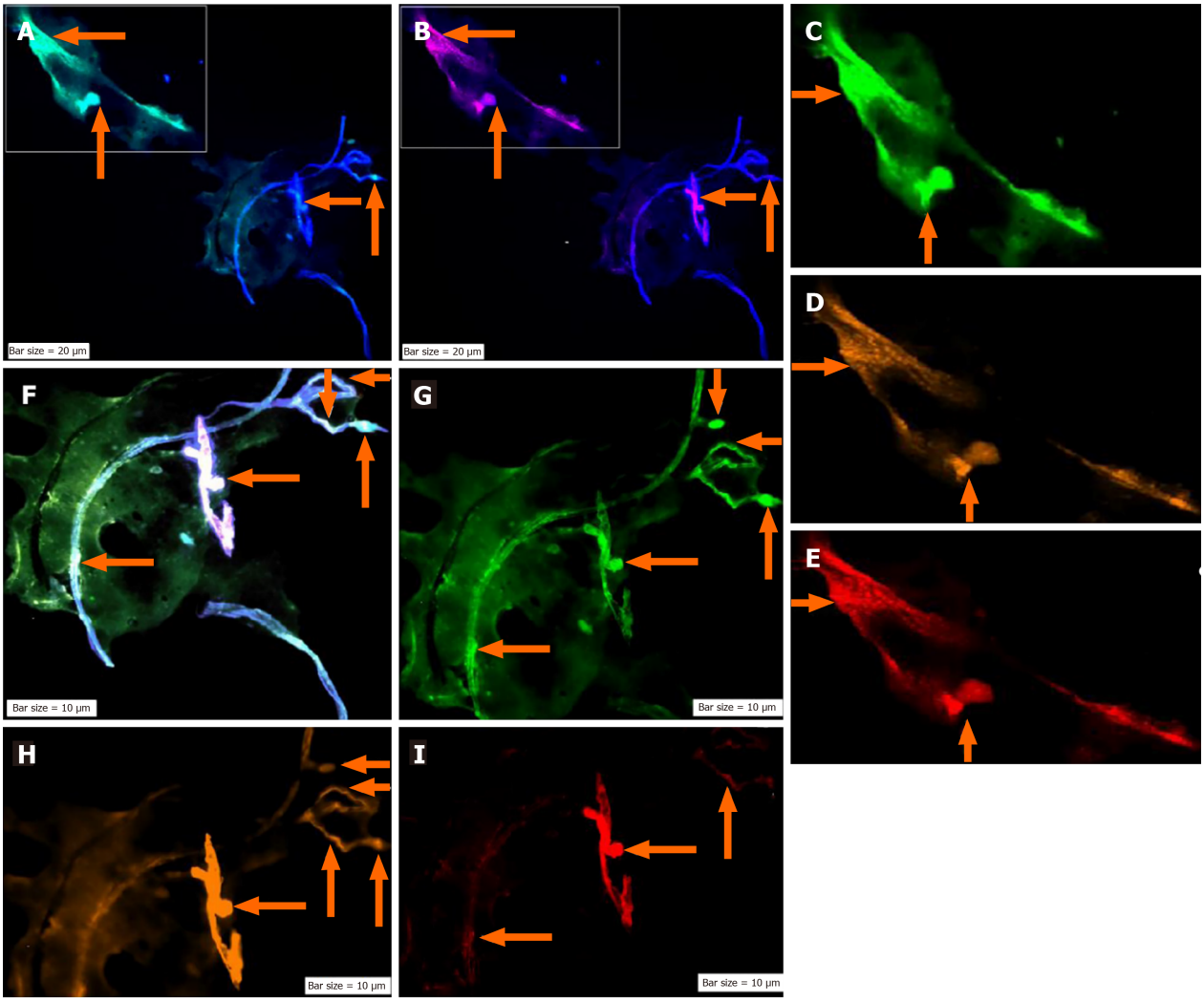
Frontal lobe functions and AD

As a major component of the cerebral cortex, the frontal lobe serves a variety of functions. It is believed that the orbitofrontal cortex (PFC) is involved in the cognitive process of decision-making and other ventromedial prefrontal cortex regions of frontal lobe; it plays a significant role in the regulation of motivation and emotion as well[24,25]. The ventromedial PFC plays a more specialized function in pleasure, happiness, and reward conditioning[26]. Executive function, however, is one of the most significant frontal lobe-related functional groups. Executive functions are typically cognitive processes that help people tackle challenging, unique, and complicated tasks by choosing and fusing actions or thoughts with internal goals and mediating activities over time[27-29]. Working memory, cognitive flexibility, and inhibition are all components of executive function, which is reliant on top-down (*i.e.*, goal-driven) control of distributed processes taking place across the brain. The nature of the processes being controlled determines the precise behavioral output[30]. Key cognitive processes relating to social, emotional, and motivational elements of conduct are carried out by prefrontal cortical areas. Working memory, goal-driven attention, task switching, planning, problem-solving, and the need for novelty are all functions of the dorsal lateral prefrontal cortex. The medial prefrontal cortex is involved in self-awareness, motivation, emotional regulation, and updating goal-directed behavior; the orbitofrontal cortex is involved in personality, inhibition, and emotional and social reasoning. The ventral lateral prefrontal cortex is involved in inhibition, response selection, and monitoring[27]. Dysexecutive syndromes have historically been linked to dorsolateral prefrontal cortex damage, but it is now understood that they can also be caused by a parietal-temporal-frontal system impairment, which is the focus of a specific type of atypical AD. Simple daily tasks requiring executive control and a variety of neuropsychological tests are among the tasks that this dysexecutive Alzheimer phenotype performs poorly on. Disrupted executive control over social, emotional, and motivational elements of behavior characterizes dysexecutive syndromes, which are more closely associated with the frontal lobe[31].

EEG findings in AD

It is crucial to take into account both the rhythms' location in the brain during EEG analysis in addition to the rhythms themselves. For instance, because alpha rhythms represent the activity of several neuronal populations, they have different neurological correlations when they are observed in the occipital, temporal, or frontal cortexes[32].

The majority of EEG studies used to diagnose AD have been based on spectral decomposition of scalp signals in both the resting state with closed eyes and open eyes. We now understand that healthy aging causes changes in brain activity, which are consequently recorded in EEG recordings. They include an increase in delta (1-4 Hz) and theta (4-8 Hz) power, a decrease in background activity, and a slowing of alpha activity (8-13 Hz)[33]. During physiological aging, the

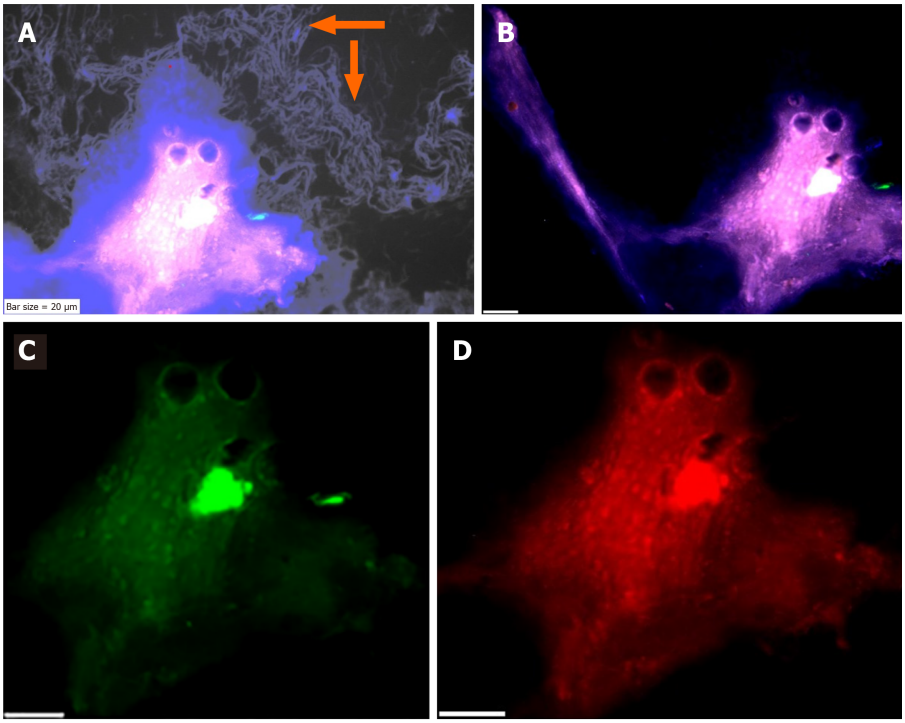


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Figure 6 Protein expression of neural marker/CD133/vascular endothelial growth factor in the circulated neural cells from the neural system to the blood stream in a patient with Alzheimer disease. A: Merged of dapi/Ne (Ne: Neural cells conjugated with dapi/Ne); B: Merged of dapi/Pe-cy5, CD133, Same cells conjugated with fluorescein isothiocyanate reflecting high expression; C: Neural cells, conjugated with fluorescein isothiocyanate (FITC)/Ne; D: Neural stem cells, conjugated with Pe-cy5 with mosaic pattern including the cells with high, very low-, and lacking-expression; E: Conjugated with Pe-cy5/neural cells; F: Co-expression of Dapi/Ne/CD133/VEGF; G: Neural cells conjugated with FITC; H: Vascular endothelial growth factor conjugated with Rpe; I: CD133 conjugated with Pe-cy5. The migrated neural cells with high or low expression are shown with arrows. The micro-vesicle and the vascular section harboring the migrated neural cells are detectable. The micro-vesicle and the vascular section harboring the migrated neural cells are detectable. Ne: Neural cells conjugated with FITC; CD133: Neural stem cells, conjugated with Pe-cy5; VEGF: Vascular endothelial growth factor, conjugated with RPe; The CNCs were explored by IF method for combination of neural marker, neural stem cell for Ne/CD133 (Figure 7).

magnitude of the alpha rhythm reduces in posterior cortical regions, and this is related to the level of general cognitive function[34]. However, numerous studies indicate that compared to age-matched controls, both mild cognitive impairment (MCI) and AD suffer from changes in the pattern of their EEG recordings: Alpha and beta rhythms typically decline, whereas delta and theta oscillations generally increase[35-37]. Such evidences highlight the key application of EEG through the course of disease.

At the AD-initiation, there is an increase in slow waves, notably in the theta rhythm. The earliest indicators of cognitive deterioration and the theta power rise are related[38]. Theta relative power, (which is expressed as the percentage of theta band power to all other bands), is higher in AD than in MCI and higher in MCI than in healthy controls and is associated with worsening performance across all cognitive domains[39]. alpha power is lower in AD than in MCI, and in MCI than in normal people[40]. There is a correlation between the decline in alpha activity and the severity of the illness and the cognitive abnormalities[40]. In addition to spectrum features, EEG also has synchronization features. EEG synchronization also describes how various brain oscillations are adjusted. When two distinct signals lock in phase, become phase-amplitude coupling, or modulate their amplitudes simultaneously (amplitude-amplitude coupling), they are said to be coupled[41]. One of these is spectral coherence measurement, which measures the spectral covariance of activity between two electrode locations. When compared to healthy controls, EEG coherence in AD participants exhibited statistically significant changes[42-44]. Alpha wave coherence in the temporo-parieto-occipital regions decreased in AD patients, although delta wave coherence between the frontal and posterior regions increased[45]. Considering the previously



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Figure 7 Protein expression of neural marker, and CD133 in the circulated neural cells from the neural system to the blood stream in a patient with Alzheimer disease. A: Merged of phase contrast/dapi/neural marker (NE)/CD133, in which the presence of beta-Amyloid is remarkable in the background of this image; B: Merged dapi/NE/CD133, reflects the co-expression between NE and CD133; C: Diverse expression of NE, reflects the dissected of only upper section of image with low expression of NE; accompanied by an isolated section with low expression of NE; including group of cells with positive expression; D: Diverse protein function including high and low expression of CD133. The protein expression of NE/vascular endothelial growth factor/erythroblastosis virus E26 oncogene homolog 2 is also provided (Figure 8).

mentioned theory that basal forebrain neurons are severely impaired, all EEG characteristics associated with AD, such as changes in frequency patterns and synchrony measurements, may be caused by the loss of neurons, altered anatomical structure of the neuronal tracts, as well as altered release of neurotransmitters, all of which lead to impairments in neural activity[44].

Highlighted points of view

Neurological disorders are extremely complicated with an influential impact on the whole society including the patients' family and close friends. Therefore, the educational packages are required to create the cooperative surrounded living atmosphere between the patients, family and the friend.

Regarding the muti-dimentional approach of the neurological disorders, including AD, the following facts and directive points of view in the recent review article provided by Tanaka and Vécsei[46]: Differentiating the "neuroprotective and neurodegenerative modules of neurological and neuropsychiatric diseases", highlighting the etiology, available techniques, diagnostic and therapeutic approaches of neurodegenerative diseases.

As the whole, neural engagements can be stabilized by the" neuroplasticity" which is characterized by: (1) The nervous system capabilities to convert other components; (2) based on the status of functions, structural format, and constructive ability; and (3) being malleable, tolerated and adaptive against the non-desirable event and challenges[46].

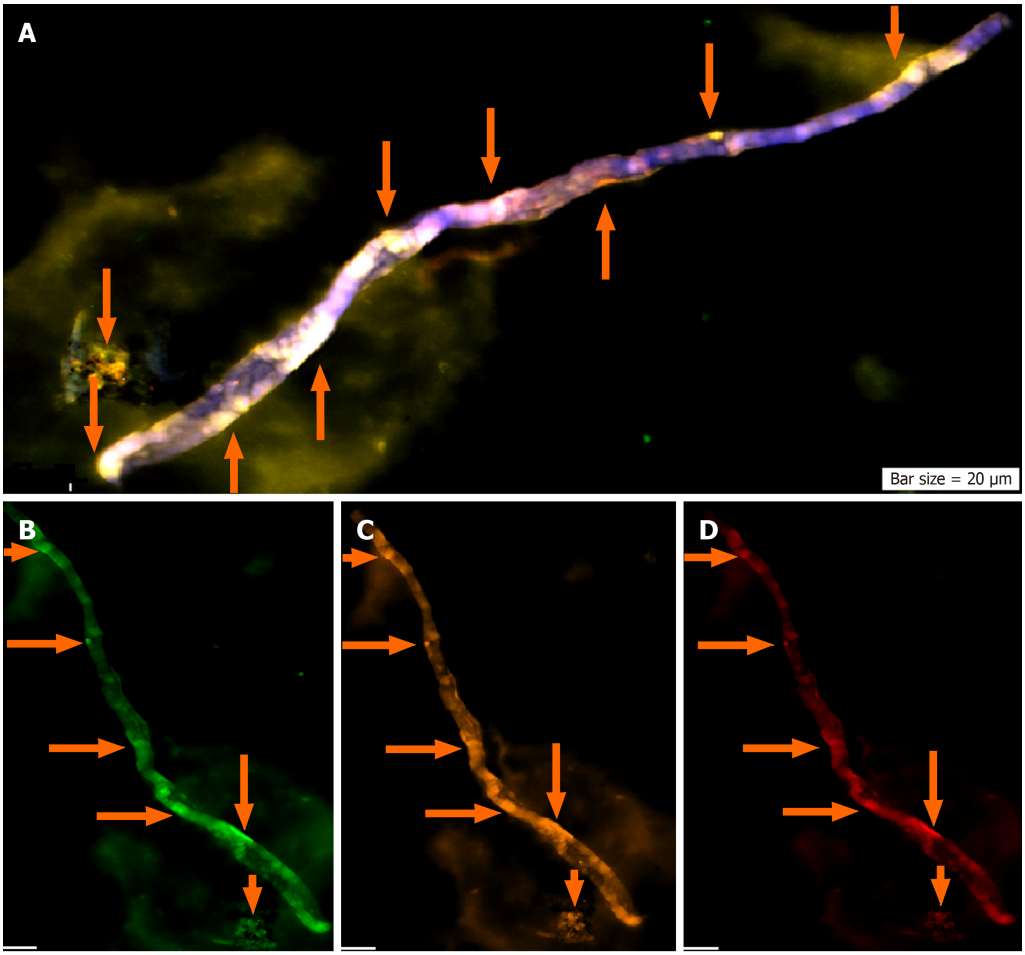
Brain EEG

Regarding the traditional brain technology for AD- patients, and by focusing on the psychological disorders, the neurodevelopmental aspects of the brain, have not been much unmasked. Indeed, EEG, as a globally trusted screening system, applied to measure the brain motion for a century. EEG affords the brain's electrical motion and measure the electro cortical rhythms[6,7,9,14].

The EEG reports are skillful in: (1) Resting status; and (2) closed eyes using a Delta-med digital EEG acquisition system for 20 min. Scalp electrodes were adjusted according to "the modified International 10 ± 20 system plus 11 additional electrodes by 256 Hz sampling rate". Thirty electrodes were positioned on the scalp include Fp1, Fp2, F7, F3, Fz, F4, F8, FT7, FC3, FC7, FC4, FT8, T3, C3, Cz, C4, T4, TP7, CP3, CPz, CP4, TP8, T5, P3, Pz, P4, T6, O1, Oz, and O2.

Furthermore, by considering especial care about the patients affected with AD at preclinical zone, information including classification up to the diagnostic principles requires the complementary attention[17].

Ratio was calculated between different brain- channels to achieve the manner of motions between different channels at pre- and post-mediation with the LM. Therefore, the LM as a balancing and relaxing the neural targets mediated the Volunteers.



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Figure 8 Protein expression of neural marker/vascular endothelial growth factor/erythroblastosis virus E26 oncogene homolog 2 in the circulated neural cells from the neural system to the blood stream in a patient with Alzheimer disease. A: Circulated neural cells (CNCs) with dapi/neural marker/vascular endothelial growth factor (VEGF)/erythroblastosis virus E26 oncogene homolog 2 (Ets2) reflecting remarkable co-epression between 3 proteins; B: Few CNCs with Ne (conjugated with fluorescein isothiocyanate), reflecting high expression; C: The cells are conjugated with RPe and reflects VEGF with diverse high-, low- expression; D: Cells are conjugated with Pe-cy5 reflects Ets2 with diverse, mostly low-, accompanied by cells with high- and lack of expression.

Complementary notes, challenges and highlights on EEG in brain: Focusing on AD

The history and the benefit of EEG in research and clinics are, approximately, related to the 120 years ago (since 1930s). However, the scientific knowledge lacks the course of evolution through the brain channels. Whatever the Scientists unmask, is required to reflect: (1) The initial/fundamental event(s) through the brain channels; (2) priority of the events; (3) the cascade manner of the alterations; (4) interaction between the channels; (5) stability of the alteration(s); (6) unmasking the resting state of EEG; and (7) unveiling the course of evolution in the human brain channels.

The function and role of Broadman's areas including 64 channels of EEG are directive to unmask the complementary/paralleled function of the brain territory. However, the interaction between the channels requires to be personalized and classified. Any kind of mediation may increase or decrease the activity of alpha and gamma waves, respectively, and affect the brain metabolism and anxiety.

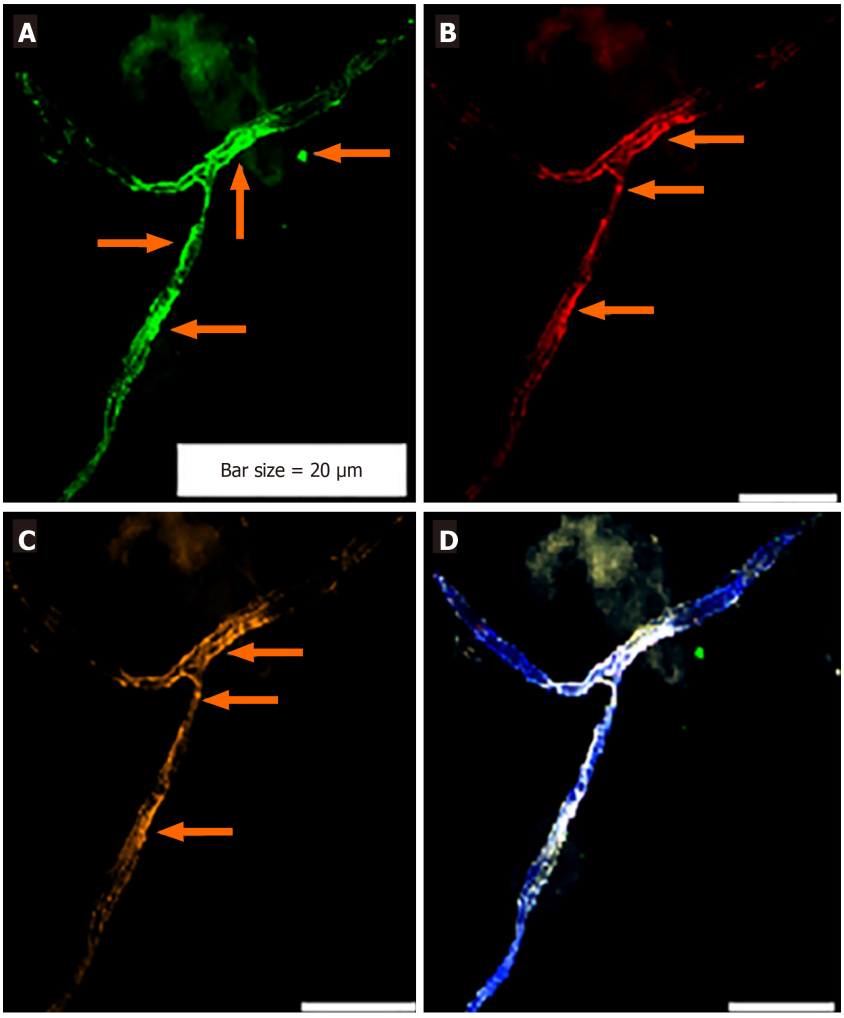
Mediation by LM led us to provide a brain channel based and personalized model, and to unmask the nature of process through the brain channels, including a significant increase in Alpha and Beta bands at frontal, central and occipital lobes of the brain.

Ratio between different brain channels unveil the diverse signal pattern at the pre- and post-mediation with the LM.

As a question, whether any mechanism relies on an interactive system between the brain's channels or not. So, 63 channels were investigated in 5 cases, at pre- and post-mediation with LM.

Based on the *t*-test, a significant increased pattern in alpha wave- and decreased in gamma wave activity, and reduction in the brain metabolism and anxiety were observed in the candidates' EEG ($P < 0.05$). As the result, a significant increase in Alpha and Beta bands of the frontal, central and occipital lobes of the brain were observed. The waves including Alpha 1,2; Beta 1-3, in both eye conditions, either open or close, the power is found between 1 to 1.6. Diversity is remarkable with close eyes than in open eyes condition.

By considering the power ratio of brain signals, the post- *vs* pre-mediation of LM was explored in the candidates. The outcomes of mediation with LM commanded to significant escalation of the power of alpha band wave at Fz, F4, FC2 sites (frontal lobe), Cz (central lobe) and O1 (occipital lobe). The increased activity of Alpha waves in Centro-frontal regions



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Figure 9 Protein expression of cytokeratin-19/CD45/endothelial growth factor in circulating neural cells in the blood stream of an apparently healthy individual under mediation with light music. A: The circulating neural cells (CNCs), conjugated with fluorescein isothiocyanate for cytokeratin 19 (CK19); B: Same image with Cy5 for endothelial growth factor (EGF); presenting less cells expressing than CK19; C: Same image with R-phycoerythrin for CD45, and expressing less than CK19; D: Co-expression of dapi/CK19/EGF/CD45. The arrow refers to the CNCs cells with remarkable expression. Presence of CNCs was confirmed by the mode of cytokeratin 19+/CD45.

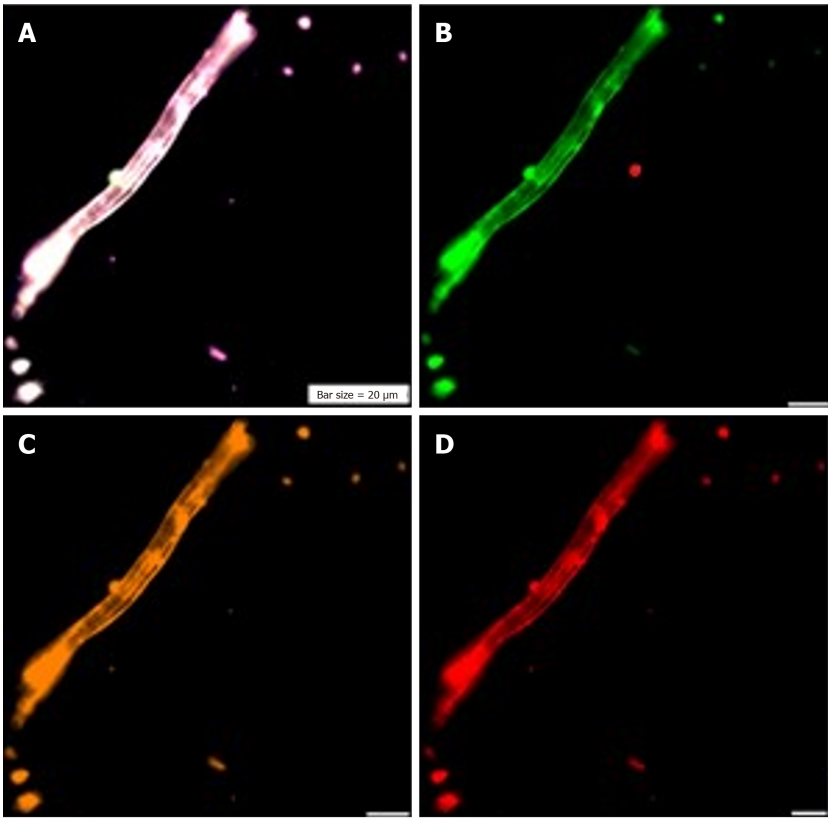
represents *edited* brain activity, metabolism, and reduced anxiety. Conclusively, the brain requires fewer properties for managing its cognitive task. Data of EEG revealed that the mediation, has significantly led to: (1) Increasing the Alpha and Beta bands of the frontal, central and occipital lobes of the brain; (2) reduction of the brain metabolism and anxiety; and (3) declining the activity of gamma wave in the EEG.

Remarkable diverse pattern was observable between absolute pattern for Gamma 1 and 2 at open eye- (characterized with < 1 absolute power) than close eye-condition (characterized with > 1, ranging between 1-7).

Status of the absolute power ratio in all brain channels sounds as an Informative panel in the brain research and with possible translational impact on personalized classification in Medical Neurology. Exploring of the absolute power ratio in all brain channels could be performed upon the physician's authorization, at different periods of life, including childhood, adulthood and elderly, by issuing the '*Brain ID card*'. Such preliminary and three complementary ID-card pave the way towards tracing the course of *evolution* at brain channels, and the probable connective pathways to the certain disorders. Such Information provides the 5xP package for any preventive, and/or the clinical recommendations to the target patients (Figures 10, 11, 12, and 13).

Conclusively

The provided Ratios are considered as the reliable indices for organizing the behavioral status of brain channels (Figures 10, 11, 12, and 13) to: (1) Guarantee an early detection; (2) being accompanied by performance of PE in the non-invasive tissues, including 2-3 mL peripheral blood, buccal smear and/or mucosa, nail, urine, stool, and/or through performing circulating cells from the target organs/tissues; (3) considering as the personalized, predictive, preventive, and prognostic panel for the patient's relatives within the pedigree; (4) cooperation between different channels with diverse interactive ratios lead to the balancing strategy for improving the life-quality and quantity of the patients with neurological disorders and an apparently normal individuals, by light nusic and any other safe, non-invasive and beneficial



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Figure 10 Circulating neural cells. A: Co-expression of Dapi/NE/CD133/vascular endothelial growth (VEG); B: Neural marker, conjugated with fluorescein isothiocyanate; C: CD133: Neural stem cell, conjugated with R-phycoerythrin; D: Vascular endothelial growth factor, conjugated with Pe-cy5. A-D images present circulating neural cells within the blood stream. Neural marker (B) reflects high expression; neural stem cell (C) and VEGF (D) are characterized with the harmonic and higher protein expression.

intervention(s); and (5) by considering the provided hypothetic based data, heterogenic/diverse results presented the occurrence of evolutionary course within the brain channels. Multi-interaction between different channels, are suggested as the personalized and constructive markers with unlimited cooperation in health and disease.

The influence of the light music on the brain channels reflected the diverse patterns in the absolute power ratio at pre- to post-intervention (Figures 10, 11, 12, and 13).

Power of the brain channels

Mean Power of Delta (HZ) before and 20 min after mediation with LM revealed the significant differences, in the following brain channels: Mean Power of the Delta waves frequency (Hz) at before and after mediation with LM is found to be significant with p-value ranging between 0.013-0.031 for the channels including Fz-, F44, FC2-,Cz- and O1-LE. Furthermore, the mean power of Beta- gamma before and after mediation ranged between 0.001-and 0.048 for the channels Fz-Le; F4-Le; FC2-Le; Cz-Le; and O1-Le of Alpha wave.

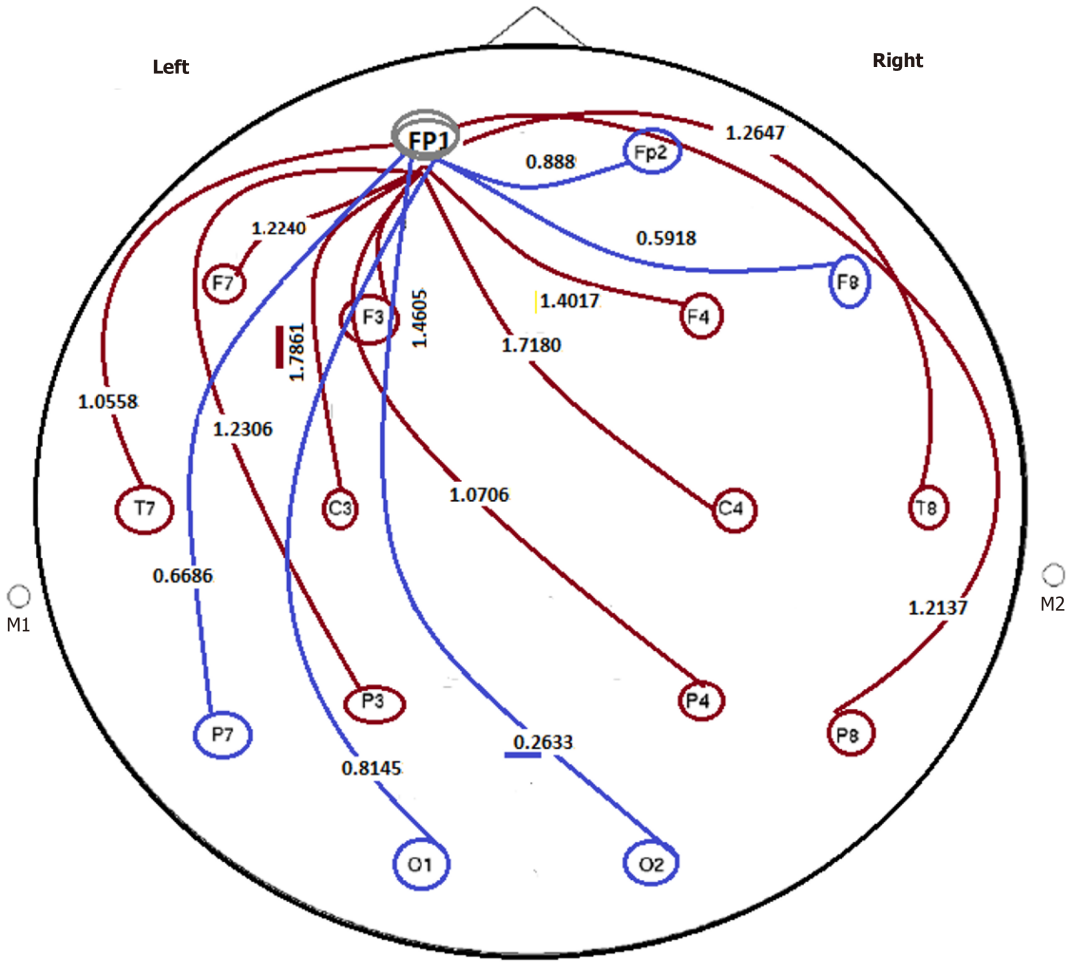
It is not documented that whether status of the delta-wave frequency, between the closed and opened eyes in different brain channels is diverse or not. Therefore, we aimed to explore the ratio-pattern between absolute powers in different channels (Figures 10, 11, 12, and 13).

Interaction between different brain channels, either balanced or imbalanced, is a personalized pattern, leading to the new classification in the brain. Exploration of EEG according to the following results and discussion reflect the remarkable diversity between different channels, which could be classified as the unique/personalized classification for the brain behavioral Science (Figures 10, 11, 12, and 13).

Amongst the current diverse status of the delta-wave frequency, 8/9 ratios are characterized with the higher frequency for the closed eyes than in the opened eyes' condition including F8, F7, FP1, T7, C4, F3, and F4. Besides, the diverse status includes the ratio with higher values in the opened eyes including Cz, F3, Fz, Pz, P4, P8, P3, O1 and O2 with less remarkable diversity, but still diversity is notable.

By considering the wave frequency of Delta, the ratio of closed eyes to the open eyes is reflective of: The most stable targets are traced in the C3 (12.23392/12.9445), T8 (CE: 6.965104/6.241952); and P7 (CE: 7.173759/OE: 7.919045) (Figure 11).

The indices as discordance based grouping with < 1 ratio, at post mediation by LM: The F3, Fz, Cz, P3, Pz, P4, P8, O1, O2 are classified as discordance grouping with ratio < 1. With the current diverse status of the Delta-wave frequencies, 8/9 ratios are characterized with the higher frequency for the closed eyes than in the opened eyes' condition including F8,



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Figure 11 Power ratio at pre- to post applied condition of closed/open eyes in an apparently healthy individual. By considering the applied condition- model as closed/opened eyes, evaluation was performed by considering the ratio of FP1 to other channels: (1) Minor group: Cooperation from polar section of brain, *i.e.*, between FP1/Fp2 = 0.888; FP1/F8 = 0.5918; FP1/O2 = 0.2633; FP1/O1 = 0.8145; FP1/P7 = 0.6686. All ratio < 1. Range of ratio: 0.2633-0.8145; (2) major group: have diverse destinations including FP1/T8 = 1.2647; FP1/P8 = 1.2137; FP1/F4=1.4017; FP1/C4 = 1.7180; FP1/P4 = 1.0706; FP1/F3 = 1.4605; FP1/C3 = 1.7861; FP1/F7 = 1.2240; FP1/P3 = 1.2306; and FP1/T7 = 1.0558. All ratio > 1. Range of ratio: 1.0558-1.7861. In this image, FP1 is the unique station as the primary initiating action within the brian channels, as octopods. There is a uique pattern as one direction from the upper to lower-section of the brain, including 10 indices less than 1 ratio (the blue lines) and 10 indices higher than 1 (brown lines). Such remarkable diversity is indicative of the well defined as two opposite directions from a unique central station towards 20 destinations. By defining the status of closed- to open eyes including > 1 as relaxation and < 1 as stressful conditions, then the challenging points are related to two distinct-outcomes. But as the matter of fact, the imposed rule is the unique major initiator, as the main station towards two categorized destinations. This regulation is considered as: (1) The correlative/single station, as major initiator/hypothesis; and (2) two distinct Ratio based categories, including > 1 and < 1 (Figure 11). The question is related to the diverse impact on the behavioral, emotional and other related physical and/or mental behaviors. Keeping the stable behavioral balances is the major aim with the reliable, harmless, and available tools such as light music and/or any relaxation method. This data reflects: (1) An EEG, personalized based and reliable categorized hypothesis; (2) with aim of detecting the behavioral characteristics of both group of individuals including an apparently healthy and individuals with either affected with Alzheimer disease, or at primary stages of AD; (3) considering an early detective strategy; and (4) organizing the prognostic, predictive, and preventive management as early as possible. F: Frontal; O: Occipital; C: Central; FC: Between F and C; AF: Between Fp and F; C3,4: Central Lobe; F3,4,7,8,z: Frontal lobe; FP1,2: Pre frontal cortex; PF3,4,7,8: Partial Lobe; O1,2: Occipital lobe; T7,8: Temporal 7,8.

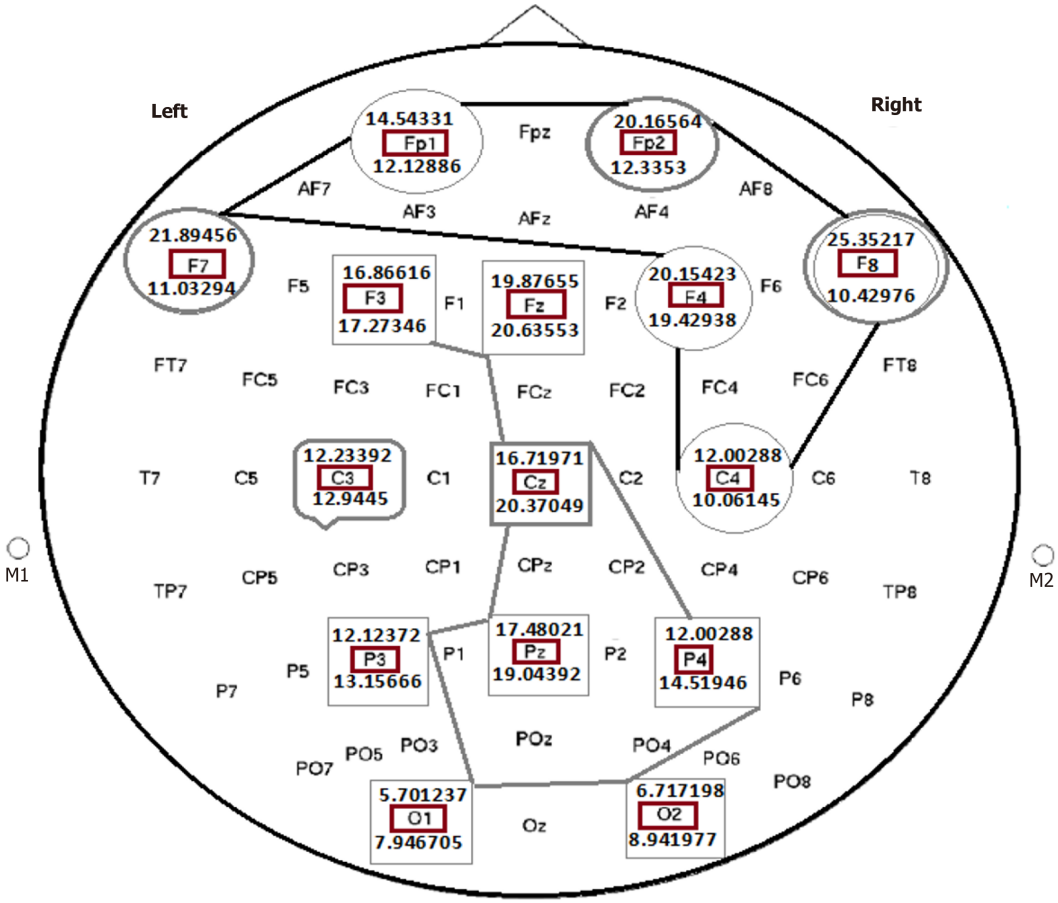
F7, FP1, T7, C4, F3, and F4. Besides, the diverse status includes the ratio with higher values in the opened eyes including Cz, F3, Fz, Pz, P4, P8, P3, O1, and O2 with less remarkable diversity (Figure 11).

By considering the wave frequency of Delta, the ratio of closed eyes to the open eyes is reflective of: The most stable targets are traced in the C3 (12.23392/12.9445), T8 (CE: 6.965104/6.241952), and P7 (CE: 7.3759/OE: 7.919045) (Figures 11 and 12).

Diverse grouping with discordance index: The indices as discordance based grouping with > 1 ratio, at post mediation by LM: F7, Fp2, F8, FP1, T7 in > 1 category, of those F8, and F7, reflect remarkable high ratio than others in this classified group (Figure 12).

Conclusively, remarkable diversity is detectable for the Ratio between closed eye/open eyes after the post mediation by LM.

Descriptive data includes before and after mediation with the LM, at the significant level of the alpha band. Mediation by LM, significantly decreases the power of Beta-Gamma band wave at points Fz, F4, FC2 (forehead areas), Cz (central



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Figure 12 Status of the absolute power Ratio by considering closed eye to open eye, based on the mediation of light music on the selected brain channels of a healthy individual. This figure includes 19 brain channels. (1) Mean power of delta (HZ) 20 min post mediation with light music; (2) channel's names represent locations of brain including Frontal (F), Occipital, central (C), between F and C, between Fp and F; and (3) the significant value: $P < 0.05$. Figure 12 is indicative of the cooperative/multi-centers/and two distinct categorized correlations including 6 and 8 destinations include: (1) Indices > 1, characterized with diverse degrees of ratio as > 1; and (2) the cooperative cluster involed 8 channels, including F3, Fz, F4, Cz, P3, Pz, P4, O1, nd O2 all with ratio < 1. These channels have impact through the middle section of brain brain from up-to down-destination. Channel C3 is characterized with almost ratio = 1, and it seems that the cooperative manner with other channels, is considered as an independent chanel. Catagories: Closed eyes (CE), opened eyes (OE); CE < OE; CE > OE; CE remarkably > OE; Almost equal CE and OE. This figure includes 19 brain channels. F: Frontal; O: Occipital; C: Central; FC: Between F and C; AF: Between Fp and F; CE: Closed eyes; OE: Opened eyes. By considering 19 channels, the absolute power ratio in all brain channels by considering close eye to open eye, based on the mediation of light music in the brain channels of a healthy individual provided the following results (Figure 13): Diverse grouping with discordance index includes: The indices as discordance based grouping with < 1 ratio, at post mediation by light music, and includes: F3, Fz, Cz, P3, Pz, P4, P8, O1, O2 which are classified as discordance grouping. The indices as concordance based grouping and includes: (1) HZ Pre- and 20 min post mediation with light music; (2) channels' names represent locations of 19 channels in the brain including: C3,4,z: Central Lobe; F3,4,7,8,z: Frontal lobe; FP1,2: Pre frontal cortex; PF3,4,7,8,z: Partial Lobe; (3) the results, based on the ratio of closed eyes to the open eyes is classified as concordance and discordance; and (4) closed eyes, and opened eyes (P value: $P < 0.05$). In upper frame, except F3 with ratio of < 1, the other 9 channels present ratio of > 1. The blue-line group, are reflective of mosaicism including P7, C3 and T8 with equal ratio; Cz with < 1, and C4 with > 1. Interestingly, the most harmonic channels are traced within the middle region (red line includes the cooperative panel) which are characterized with the < 1 ratio between 9 different channels through the horizontal and vertical distributed channels (Figure 13).

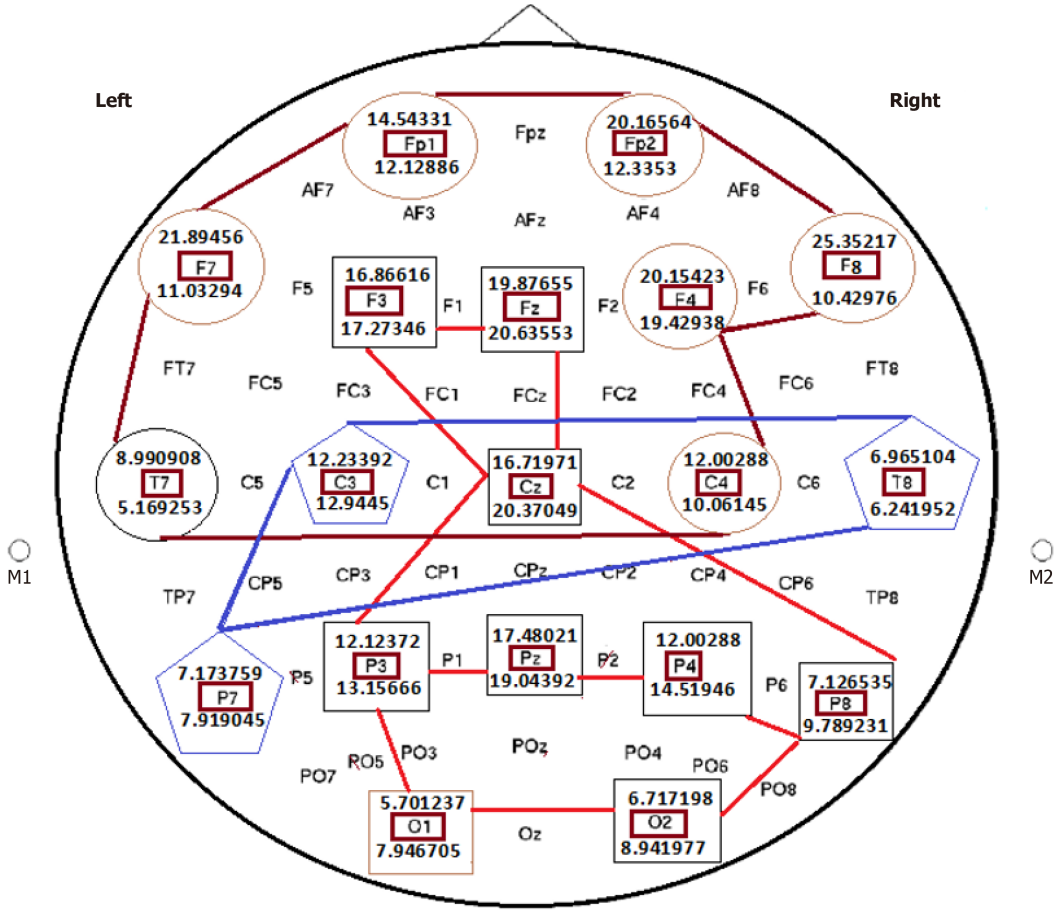
region) and O1 (occipital region of the brain). Such a decreased power of beta-gamma reflects the reduced activity and connectivity of brain between frontal/central regions.

Concisely, an increased alpha power and decreased beta-gamma power after mediation of the LM is remarkable. This link between neural oscillations in diverse frequency bands proposed to harmonize neural processing.

Furthermore, concordance and discordance between different channels of brain is the 5xP model after mediation with LM or any selected mediated factor, upon the specialists/physician's recommendation for further planned clinical management(s).

CONCLUSION

Application of CNCs and correlated Ratio based between brain channels by providing the 5xP personalized clinical management model for early detection and therapy of the patients with AD and their targeted/predisposed relatives. Highlights offer an early detective platform by considering neuro-genetics/cell biology, CNCs; molecular investigation,



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Figure 13 Status of the absolute power Ratio in all brain channels by considering close eye to open eye, based on the mediation of light music in the brain channels of a healthy individual. This figure includes 19 brain channels. The absolute power ratio in all channels are an Informative frame in the brain research with target based translational impact on personalized classification in Medicine/Neulogy. The cooperative strategy between different channels is rather complicated in which 3 diverse, unique and cooperative platforms are established which could optimize the status of the mental behavior and health status of the target individuals. Brain is governed by multi-centered channels, being capable of influential cooperation.

for the predisposed individuals to AD- or other neural disease. The initiation and functional stage of triangle PE including neural-marker, stem cell (CD133), and VEGF is unmasked not only at the embryonic phase, but through the beginning of chorionic villus period around 8th-10th of gestational weeks. The mediation with LM resulted to remarkable escalation of the power of alpha band wave at frontal lobe including Fz, F4, FC2 sites, Cz in central lobe and O1 in occipital lobe and the increased activity of Alpha waves in Centro-frontal regions.

ARTICLE HIGHLIGHTS

Research background

Circulating neural cells (CNCs) is an essential strategy which offers the *pentagonal* application including the Personalized, Prognostics, Predictive, Preventive, predisposing (5xP) panel in the clinical neurology and Neuro-Science. Therefore, it was aimed to explore the developing picture of CNCs' behavior of the brain cells in the blood stream. CNCs are an available approach for the preventive Medicine and the personalized/target-based therapy.

Research motivation

This strategy includes and offers: (1) Routinizing the CNCs assay through the high and reliable enumeration of the CNCs; (2) providing the required information's of CNCs to the neurological clinics for the patients affected with neurological disorders including Alzheimer disease (AD); (3) providing adequate Information on the application of CNCs for the protocol to the target clinical centers for either their referral patients affected with, or predisposed to AD as promptly as possible; (4) highlighting the safety, rapidness of the process, non-invasiveness of the CNCs exploration; and an early/preventive strategic detection of the AD in the probands and the relatives through their pedigree; and (5) considering the pedigree-based analysis for tracing of any micro- and macro-environmental predisposing factors, including nutrition and the history of the neurological based diseases including AD.

Research objectives
In the Alzheimer-prone families, the history of any related clinical sign of the neurological symptoms including light and/or progressive through different generations of the referral suspicious case, could be candidate for exploring CNCs. If there is any persuasive micro-and/or macro-environmental risks, the target of the proband's offspring may be candidate for the CNCs.

Research methods
The originality of the provided research is based on the Manuel performance and exploration of the single cell based analysis with high enumeration. This strategy is capable to unmask the heterogeneity, diversity and highlights the specific role of the target proteins, and identification of the novel involvement of additional protein through the developmental stages of AD. Furthermore, by respecting the bridging system between Medicine and Science, and according to the primary results of Electroencephalography, as a golden performance, an innovative, complementary and predictive model is provided in this paper. Finally, based on an early detection, and the developmental process through the passed progressive period of AD, the personalized detection, hopefully, leads to the personalized therapy.

Research results
The novel results of the provided manuscript are characterized with: Personalized data; Single cell based strategy; and translatable data for the index case and their relatives; an early detection of the protein expression (PE) and brain channels bridging systems, the personalized therapy, could be translated, either for Alzheimer or the related psychological related to the brain disorders. Hypothetic/heterogenic/diverse results highlight evolutionary course within the brain channels.

Research conclusions
Unmasking the diverse pattern of PE of the migrated cells from brain to the blood stream, by an adequate enumeration of the CNCs based on the Manuel analytical approach. Unveil the heterogenic pattern of the analyzed target proteins' expression. Multi-interaction between brain channels, are considered as the personalizd/constructive markers with unlimited cooperation in health and disease.

Research perspectives
Unmasking the role of other candidate protein(s). Considering the informative publication(s) as the complementary educational package. Bridging between Science and Medicine by considering the pedigree based translational system for neuro-model, as an early detective platform. Suggesting for stablishing the Neuro-clinic with the aims including 'Personalized-Predictive/Early detection/Preventive clinic for the families with 'Alzheimer disease' and in future with neurogenetics disorders'.

FOOTNOTES

Author contributions: Mehdipour P designed the research, analytical strategy and contributed the data on genetics and cell biology; Fathi N and Nosratabadi M, and Mehdipour P contributed equally to this work and analysed data of the brain channels; all authors wrote, have read, and approved the final manuscript.

Institutional review board statement: The study was reviewed and approved by investigation of the fundamental pattern in the brain channels' EEG of five apparently healthy individuals, with ethics ID "IR.IUMS.REC.1399.310".

Institutional animal care and use committee statement: This study was not involved any animal experiments.

Informed consent statement: All study participants or their legal guardian provided informed written consent about personal and medical data collection prior to study enrolment.

Conflict-of-interest statement: The authors declare that they have no conflict of interest.

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

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Country/Territory of origin: Iran

ORCID number: Parvin Mehdipour 0000-0001-8358-263X; Nima Fathi 0009-0006-6780-6908; Masoud Nosratabadi 0000-0001-9080-9070.

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REFERENCES

1 **Alzheimer A**, Stelzmann RA, Schnitzlein HN, Murtagh FR. An English translation of Alzheimer's 1907 paper, "Uber eine eigenartige Erkankung der Hirnrinde". *Clin Anat* 1995; **8**: 429-431 [PMID: 8713166 DOI: 10.1002/ca.980080612]
2 **Pastorcic M**, Das HK. Regulation of transcription of the human presenilin-1 gene by Ets transcription factors and the p53 protooncogene. *J Biol Chem* 2000; **275**: 34938-34945 [PMID: 10942770 DOI: 10.1074/jbc.M005411200]
3 **Zeng H**, Sanes JR. Neuronal cell-type classification: challenges, opportunities and the path forward. *Nat Rev Neurosci* 2017; **18**: 530-546 [PMID: 28775344 DOI: 10.1038/nrn.2017.85]
4 **Stam CJ**. Use of magnetoencephalography (MEG) to study functional brain networks in neurodegenerative disorders. *J Neurol Sci* 2010; **289**: 128-134 [PMID: 19729174 DOI: 10.1016/j.jns.2009.08.028]
5 **Fernández A**, Zuluaga P, Abásolo D, Gómez C, Serra A, Méndez MA, Hornero R. Brain oscillatory complexity across the life span. *Clin Neurophysiol* 2012; **123**: 2154-2162 [PMID: 22647457 DOI: 10.1016/j.clinph.2012.04.025]
6 **Porcaro C**, Balsters JH, Mantini D, Robertson IH, Wenderoth N. P3b amplitude as a signature of cognitive decline in the older population: An EEG study enhanced by Functional Source Separation. *Neuroimage* 2019; **184**: 535-546 [PMID: 30248455 DOI: 10.1016/j.neuroimage.2018.09.057]
7 **Stam CJ**, de Haan W, Daffertshofer A, Jones BF, Manshanden I, van Cappellen van Walsum AM, Montez T, Verbunt JP, de Munck JC, van Dijk BW, Berendse HW, Scheltens P. Graph theoretical analysis of magnetoencephalographic functional connectivity in Alzheimer's disease. *Brain* 2009; **132**: 213-224 [PMID: 18952674 DOI: 10.1093/brain/awn262]
8 **Maestú F**, Campo P, Del Río D, Moratti S, Gil-Gregorio P, Fernández A, Capilla A, Ortiz T. Increased biomagnetic activity in the ventral pathway in mild cognitive impairment. *Clin Neurophysiol* 2008; **119**: 1320-1327 [PMID: 18400555 DOI: 10.1016/j.clinph.2008.01.105]
9 **López-Sanz D**, Bruña R, Garcés P, Martín-Buro MC, Walter S, Delgado ML, Montenegro M, López Higes R, Marcos A, Maestú F. Functional Connectivity Disruption in Subjective Cognitive Decline and Mild Cognitive Impairment: A Common Pattern of Alterations. *Front Aging Neurosci* 2017; **9**: 109 [PMID: 28484387 DOI: 10.3389/fnagi.2017.00109]
10 **Wolvetang EJ**, Bradfield OM, Hatzistavrou T, Crack PJ, Busciglio J, Kola I, Hertzog PJ. Overexpression of the chromosome 21 transcription factor Ets2 induces neuronal apoptosis. *Neurobiol Dis* 2003; **14**: 349-356 [PMID: 14678752 DOI: 10.1016/s0969-9961(03)00107-4]
11 **Mehdipour P**, Javan J, Estiar MA, Khaleghian M, Novin N, Zaeim G, Kohan H, Akhondzadeh S. Protein Expression of ETS2 in Alzheimer's Disease and Down's syndrome as a Personalized Insight: One Gene, One Feature in Common, Diverse-Function-and Diseases. *SRL Neuro Neurosur* 2017; **3**: 001-007 [DOI: 10.3233/cbm-160546]
12 **Mehdipour P**, Javan F, Jouibari MF, Khaleghi M, Mehrazin M. Evolutionary model of brain tumor circulating cells: Cellular galaxy. *World J Clin Oncol* 2021; **12**: 13-30 [PMID: 33552936 DOI: 10.5306/wjco.v12.i1.13]
13 **Mehdipour P**, Javan F, Faghih-Jouibari M, Khaleghi M. Diversity in tumor territory of meningioma: Protein expression in vascular endothelial growth factor and epidermal growth factor. *Iran J Neurol* 2019; **18**: 43-49 [PMID: 31565199]
14 **Buzsáki G**, Anastassiou CA, Koch C. The origin of extracellular fields and currents--EEG, ECoG, LFP and spikes. *Nat Rev Neurosci* 2012; **13**: 407-420 [PMID: 22595786 DOI: 10.1038/nrn3241]
15 **Alix-Panabières C**, Pantel K. Challenges in circulating tumour cell research. *Nat Rev Cancer* 2014; **14**: 623-631 [PMID: 25154812 DOI: 10.1038/nrc3820]
16 **Jelski W**, Mroczko B. Molecular and Circulating Biomarkers of Brain Tumors. *Int J Mol Sci* 2021; **22** [PMID: 34210107 DOI: 10.3390/ijms22137039]
17 **Dubois B**, Hampel H, Feldman HH, Scheltens P, Aisen P, Andrieu S, Bakardjian H, Benali H, Bertram L, Blennow K, Broich K, Cavedo E, Crutch S, Dartigues JF, Duyckaerts C, Epelbaum S, Frisoni GB, Gauthier S, Genthon R, Gouw AA, Habert MO, Holtzman DM, Kivipelto M, Lista S, Molinuevo JL, O'Bryant SE, Rabinovici GD, Rowe C, Salloway S, Schneider LS, Sperling R, Teichmann M, Carrillo MC, Cummings J, Jack CR Jr; Proceedings of the Meeting of the International Working Group (IWG) and the American Alzheimer's Association on "The Preclinical State of AD"; July 23, 2015; Washington DC, USA. Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. *Alzheimers Dement* 2016; **12**: 292-323 [PMID: 27012484 DOI: 10.1016/j.jalz.2016.02.002]
18 **World Alzheimer Report 2015**. The Global Impact of Dementia: An analysis of prevalence, incidence, cost and trends. Alzheimer's disease International. 2015. Available from: https://www.alzint.org/resource/world-alzheimer-report-2015/
19 **Dauphinot L**, Lyle R, Rivals I, Dang MT, Moldrich RX, Golfier G, Ettwiller L, Toyama K, Rossier J, Personnaz L, Antonarakis SE, Epstein CJ, Sinet PM, Potier MC. The cerebellar transcriptome during postnatal development of the Ts1Cje mouse, a segmental trisomy model for Down syndrome. *Hum Mol Genet* 2005; **14**: 373-384 [PMID: 15590701 DOI: 10.1093/hmg/ddi033]
20 **Lyle R**, Gehrig C, Neergaard-Henrichsen C, Deutsch S, Antonarakis SE. Gene expression from the aneuploid chromosome in a trisomy mouse model of down syndrome. *Genome Res* 2004; **14**: 1268-1274 [PMID: 15231743 DOI: 10.1101/gr.2090904]
21 **Saran NG**, Pletcher MT, Natale JE, Cheng Y, Reeves RH. Global disruption of the cerebellar transcriptome in a Down syndrome mouse model. *Hum Mol Genet* 2003; **12**: 2013-2019 [PMID: 12913072 DOI: 10.1093/hmg/ddg217]
22 **Rachidi M**, Lopes C. Mental retardation in Down syndrome: from gene dosage imbalance to molecular and cellular mechanisms. *Neurosci Res* 2007; **59**: 349-369 [PMID: 17897742 DOI: 10.1016/j.neures.2007.08.007]
23 **Cimenser A**, Hempel E, Travers T, Strozewski N, Martin K, Malchano Z, Hajós M. Sensory-Evoked 40-Hz Gamma Oscillation Improves Sleep and Daily Living Activities in Alzheimer's Disease Patients. *Front Syst Neurosci* 2021; **15**: 746859 [PMID: 34630050 DOI: 10.3389/fnsys.2021.746859]
24 **Damasio AR**. Descartes' error and the future of human life. *Sci Am* 1994; **271**: 144 [PMID: 7939563 DOI: 10.1038/scientificamerican1094-144]
25 **Anderson SW**, Barrash J, Bechara A, Tranel D. Impairments of emotion and real-world complex behavior following childhood- or adult-onset damage to ventromedial prefrontal cortex. *J Int Neuropsychol Soc* 2006; **12**: 224-235 [PMID: 16573856 DOI: 10.1017/S1355617706060346]

26 **Kringelbach ML**, Berridge KC. Towards a functional neuroanatomy of pleasure and happiness. *Trends Cogn Sci* 2009; **13**: 479-487 [PMID: 19782634 DOI: 10.1016/j.tics.2009.08.006]

27 **MacDonald AW 3rd**, Cohen JD, Stenger VA, Carter CS. Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control. *Science* 2000; **288**: 1835-1838 [PMID: 10846167 DOI: 10.1126/science.288.5472.1835]

28 **Fuster JM**. The prefrontal cortex--an update: time is of the essence. *Neuron* 2001; **30**: 319-333 [PMID: 11394996 DOI: 10.1016/s0896-6273(01)00285-9]

29 **Miller EK**, Cohen JD. An integrative theory of prefrontal cortex function. *Annu Rev Neurosci* 2001; **24**: 167-202 [PMID: 11283309 DOI: 10.1146/annurev.neuro.24.1.167]

30 **Alvarez JA**, Emory E. Executive function and the frontal lobes: a meta-analytic review. *Neuropsychol Rev* 2006; **16**: 17-42 [PMID: 16794878 DOI: 10.1007/s11065-006-9002-x]

31 **Jones DT**, Graff-Radford J. Executive Dysfunction and the Prefrontal Cortex. *Continuum (Minneap Minn)* 2021; **27**: 1586-1601 [PMID: 34881727 DOI: 10.1212/CON.0000000000001009]

32 **Asadzadeh S**, Yousefi Rezaii T, Beheshti S, Delpak A, Meshgini S. A systematic review of EEG source localization techniques and their applications on diagnosis of brain abnormalities. *J Neurosci Methods* 2020; **339**: 108740 [PMID: 32353472 DOI: 10.1016/j.jneumeth.2020.108740]

33 **Ishii R**, Canuet L, Aoki Y, Hata M, Iwase M, Ikeda S, Nishida K, Ikeda M. Healthy and Pathological Brain Aging: From the Perspective of Oscillations, Functional Connectivity, and Signal Complexity. *Neuropsychobiology* 2017; **75**: 151-161 [PMID: 29466802 DOI: 10.1159/000486870]

34 **Babiloni C**, Binetti G, Cassarino A, Dal Forno G, Del Percio C, Ferreri F, Ferri R, Frisoni G, Galderisi S, Hirata K, Lanuzza B, Miniussi C, Mucci A, Nobili F, Rodriguez G, Luca Romani G, Rossini PM. Sources of cortical rhythms in adults during physiological aging: a multicentric EEG study. *Hum Brain Mapp* 2006; **27**: 162-172 [PMID: 16108018 DOI: 10.1002/hbm.20175]

35 **Kowalski JW**, Gawel M, Pfeffer A, Barcikowska M. The diagnostic value of EEG in Alzheimer disease: correlation with the severity of mental impairment. *J Clin Neurophysiol* 2001; **18**: 570-575 [PMID: 11779971 DOI: 10.1097/00004691-200111000-00008]

36 **van der Hiele K**, Vein AA, Reijntjes RH, Westendorp RG, Bollen EL, van Buchem MA, van Dijk JG, Middelkoop HA. EEG correlates in the spectrum of cognitive decline. *Clin Neurophysiol* 2007; **118**: 1931-1939 [PMID: 17604688 DOI: 10.1016/j.clinph.2007.05.070]

37 **Lizio R**, Del Percio C, Marzano N, Soricelli A, Yener GG, Başar E, Mundi C, De Rosa S, Triggiani AI, Ferri R, Arnaldi D, Nobili FM, Cordone S, Lopez S, Carducci F, Santi G, Gesualdo L, Rossini PM, Cavedo E, Mauri M, Frisoni GB, Babiloni C. Neurophysiological assessment of Alzheimer's disease individuals by a single electroencephalographic marker. *J Alzheimers Dis* 2016; **49**: 159-177 [PMID: 26444753 DOI: 10.3233/JAD-143042]

38 **Prichep LS**, John ER, Ferris SH, Reisberg B, Almas M, Alper K, Cancro R. Quantitative EEG correlates of cognitive deterioration in the elderly. *Neurobiol Aging* 1994; **15**: 85-90 [PMID: 8159266 DOI: 10.1016/0197-4580(94)90147-3]

39 **Jeong J**. EEG dynamics in patients with Alzheimer's disease. *Clin Neurophysiol* 2004; **115**: 1490-1505 [PMID: 15203050 DOI: 10.1016/j.clinph.2004.01.001]

40 **Lejko N**, Larabi DI, Herrmann CS, Aleman A, Ćurčić-Blake B. Alpha Power and Functional Connectivity in Cognitive Decline: A Systematic Review and Meta-Analysis. *J Alzheimers Dis* 2020; **78**: 1047-1088 [PMID: 33185607 DOI: 10.3233/JAD-200962]

41 **Pikovsky A**, Rosenblum M, Kurths J. Synchronization: a universal concept in nonlinear science. American Association of Physics Teachers; 2002. Available from: assEts.cambridge.org/97805215/92857/frontmatter/9780521592857_frontmatter.pdf

42 **Wang R**, Wang J, Yu H, Wei X, Yang C, Deng B. Power spectral density and coherence analysis of Alzheimer's EEG. *Cogn Neurodyn* 2015; **9**: 291-304 [PMID: 25972978 DOI: 10.1007/s11571-014-9325-x]

43 **Handayani S**, Diansari Y, Bahar E, Novantina W. EEG changes in patients with intracranial tumors and seizures symptom at Mohammad Hoesin Hospital Palembang. *J Phys Conf Ser* 2019; **1246**: 012014 [DOI: 10.1088/1742-6596/1246/1/012014]

44 **Monllor P**, Cervera-Ferri A, Lloret MA, Esteve D, Lopez B, Leon JL, Lloret A. Electroencephalography as a Non-Invasive Biomarker of Alzheimer's Disease: A Forgotten Candidate to Substitute CSF Molecules? *Int J Mol Sci* 2021; **22** [PMID: 34639229 DOI: 10.3390/ijms221910889]

45 **Locatelli T**, Cursi M, Liberati D, Franceschi M, Comi G. EEG coherence in Alzheimer's disease. *Electroencephalogr Clin Neurophysiol* 1998; **106**: 229-237 [PMID: 9743281 DOI: 10.1016/s0013-4694(97)00129-6]

46 **Tanaka M**, Vécsei L. Editorial of Special Issue 'Dissecting Neurological and Neuropsychiatric Diseases: Neurodegeneration and Neuroprotection'. *Int J Mol Sci* 2022; **23** [PMID: 35805990 DOI: 10.3390/ijms23136991]

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LETTER TO THE EDITOR

Ophthalmologic implications to consider when using hydroxychloroquine to treat COVID-19 and induced arthritis

Marco Zeppieri

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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

As the world continues to grapple with the novel coronavirus [coronavirus disease 2019 (COVID-19)], many treatments have been proposed to help alleviate the symptoms and reduce the mortality rate. Hydroxychloroquine (HCQ) is an antimalarial drug that is typically used for several autoimmune, rheumatic, and dermatological conditions. It has also been considered to treat and prevent COVID-19 and subsequent arthritis associated with the infection. This drug is known to cause retinal toxicity, which can lead to vision impairment or loss. While the exact mechanism is not yet fully understood, it is thought to be due to the accumulation of the drug in the retinal pigment epithelium. The risk of toxicity increases with long-term use or with high doses of the drug and is more likely to occur in patients with pre-existing retinal diseases or those who are predisposed to retinal diseases. In this context, several steps can be taken to monitor and minimize the risk of ophthalmological adverse events when using HCQ to treat patients with COVID-19.

Key Words: COVID-19; Hydroxychloroquine; SARS-CoV-2; Retinopathy; Maculopathy; Post-COVID-19 arthritis

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Core Tip: Hydroxychloroquine (HCQ) is typically considered when treating rheumatic and autoimmune diseases. It has been currently considered to help treat symptoms of coronavirus disease 2019 and to help alleviate several clinical manifestations after infection. In this letter, several ophthalmological implications that should be taken into consideration when using this drug are discussed. While the drug may be beneficial in treating symptoms, ophthalmological manifestations can be of clinical importance. Proper diagnoses, periodic testing, and correct management of patients in chronic treatment with HCQ can ensure that any potential ophthalmological side effects are minimized.

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TO THE EDITOR

The paper by Bajpai *et al*[1] reports an interesting thorough review of the use of hydroxychloroquine (HCQ) and azithromycin therapy to treat and prevent coronavirus disease 2019 (COVID-19). HCQ is a synthetic derivative of quinine. The drug works by inhibiting the growth of the virus, thus reducing the severity of clinical manifestations of the disease. HCQ binds to the viral protein responsible for replica-ting the virus and preventing the virus from replicating[2]. It has been found to be effective in treating the symptoms of COVID-19, including fever, cough, and difficulty breathing [3]. In addition, HCQ also has anti-inflammatory properties, which can help reduce inflammation caused by the virus. A recent commentary by Swarnakar *et al*[4] based on the review by Bajpai *et al*[1] presented the possible interesting use of HCQ to treat arthritis induced after viral infection. Numerous studies in the literature have shown the benefits offered by HCQ in alleviating debilitating symptoms. The aim of this letter article is to summarize the important ophthalmologic considerations when HCQ is considered in the treatment of COVID-19. The issues regarding whether or not HCQ is effective in treating COVID-19, which was reported in the papers recently published in this journal[1,4], will not be considered in this article. Here, the important potential risk factors and ophthalmological side effects are briefly mentioned, with the aim of reminding clinicians about the ophthalmological considerations and best practices to monitor, diagnose, and manage patients using HCQ. This is imperative to ensure that any potential ophthalmological side effects and toxic damage are minimized.

HCQ is a drug traditionally used to treat and prevent a variety of different conditions. In brief, it is an antimalarial and anti-inflammatory medication that works by inhibiting the growth and spread of certain parasites, bacteria, and viruses. At the molecular level, HCQ works by inhibiting the activity of certain enzymes known as heme polymerases[5]. These enzymes are responsible for the synthesis of heme, a molecule that is essential for the development of some parasites, bacteria, and viruses. By blocking the activity of heme polymerases, HCQ prevents the growth and spread of certain parasites, bacteria, and viruses. HCQ is used to treat a variety of conditions, including malaria, lupus, and rheu-matoid arthritis[6]. In malaria, it is used to prevent and treat the disease, while in lupus and rheumatoid arthritis, it is used to reduce inflammation and relieve symptoms. In addition, HCQ is also used to treat and prevent certain types of malaria.

HCQ is generally considered to be safe and well tolerated, but some people may experience systemic side effects such as nausea, dizziness, and headache. Studies have shown that HCQ can sometimes cause glucose abnormalities, cardiotoxicity (conduction abnormalities, cardiovascular collapse, cardio-myopathy, *etc.*), gastrointestinal effects, neuromyotoxicity, neuropsychiatric events, and dermatologic reactions[7].

There are several important ophthalmologic effects of HCQ[8]. This drug has been shown to influence cellular autophagy and lysosomal activity. HCQ can also interact with membrane stability and alter transcriptional activity and signaling pathways[9]. The ocular side effects include corneal deposits, retinal pigmentary changes, maculopathy, and optic neuritis. These complications need to be closely monitored and managed in patients using high doses and long-term therapy with HCQ. Differential diagnosis, alternative therapies for underlying disorders, proper periodic testing for functional and anatomical toxicity, and treatment options are imperative to limit drug toxicity in patients under chronic HCQ medication.

Corneal deposits are usually seen in patients taking HCQ for more than 5 years and are generally considered benign. Corneal deposits and damage induced by HCQ can vary depending on the dosage and duration of treatment. Damage to the cornea can manifest in the form of corneal opacity and corneal deposits. These deposits are typically referred to as corneal verticillata and are comprised of amor-phous, yellow-white deposits scattered throughout the cornea[10]. Corneal verticillata can cause significant visual impairment if they are dense enough to obscure vision. The mechanism of action by which HCQ induces corneal deposits is not fully understood. It is believed that HCQ accumulates in the corneal epithelium and forms a complex with proteins and lipids, resulting in the formation of deposits[11]. This could also possibly be due to reduced tear turnover and accumulation of the drug in the tear film. Additionally, it is thought that HCQ can interfere with the normal metabolism of the cornea, resulting in the accumulation of lipids and other deposits. The effects of HCQ on the cornea can be severe and can lead to permanent vision loss. Therefore, it is important to monitor patients taking HCQ for the development of corneal deposits and damage. If corneal deposits are found, HCQ should be discontinued and other treatments can be considered.

Retinal pigmentary changes are characterized by granular deposits at the level of the retinal pigment epithelium (RPE) and can lead to decreased visual acuity and progressive visual field loss[12]. Maculo-pathy is a form of retinopathy that is characterized by a bull's eye maculopathy, which is a specific pattern of retinal damage that specifically affects the circular area of damage to the macula. This small area is the central part of the retina responsible for sharp, central vision [13]. The mechanism of bull's eye maculopathy due to HCQ toxicity is not completely understood, but it is thought to involve the drug's effects on the RPE cells due to the accumulation of the drug in the RPE, leading to the formation of pigmentary changes. These RPE cells play a key role in supporting the function of the photoreceptor cells in the retina, and HCQ may disrupt this support system, leading to damage to the photoreceptor cells and the characteristic bull's eye pattern of damage. This can lead to decreased visual acuity and pro-gressive visual field loss.

Diagnosis of bull's eye maculopathy due to HCQ toxicity can be challenging, as it can be difficult to distinguish from other retinal disorders. Differential diagnosis includes other forms of macular degeneration, such as age-related macular degeneration and Stargardt disease, as well as other retinal disorders such as cone dystrophy and macular dystrophy[14]. Testing for HCQ toxicity includes complete ophthalmologic examination with vision testing and dilated fundus assessment, visual field testing, spectral domain optical coherence tomography, fundus autofluorescence imaging, and multi-focal electroretinography[15].

Optic neuritis is an inflammatory disorder of the optic nerve, which can lead to reduced visual acuity, decreased color vision, and decreased peripheral vision. This complication due to HCQ is very rare. Differential diagnosis can be made by magnetic resonance imaging, angiography, blood tests, visual field testing, and visual evoked responses[16]. The mechanism of action is thought to be due to an autoimmune response to the drug, leading to inflammation of the optic nerve[17].

The incidence of HCQ toxicity is dose-dependent, with higher doses and longer duration of treatment increasing the risk of toxicity[18]. The recommended daily dose of HCQ that is generally considered safe is up to 6.5 mg/kg/d, but at higher doses, the risk of toxicity increases. Risk factors include a cumula-tive dose of > 1000 g of HCQ, treatment duration of more than 5 years, preexisting liver or renal dysfunction, being very elderly, and preexisting retinopathy[19].

It is yet unclear how HCQ can cause retinal damage. According to studies, the medication impacts the metabolism of retinal cells and binds to melanin in the RPE, which may help to explain why some people continue to experience side effects even after stopping the prescription. With regards to dysfunction related to toxicity, it is thought that HCQ binds to melanin in the RPE, blocking its function, which can lead to irreversible photoreceptor loss and resulting visual field defects over the afflicted sector of the retina. In some cases, bull's eye configuration can be seen as a ring scotoma on a visual field test when RPE malfunction leading to atrophy occurs across the perifoveal ring when the central fovea is spared. The half-life of HCQ is about 1 mo, with a washout period of about 6 mo. Early diagnosis of HCQ retinal toxicity is crucial to prevent maculopathy from progressing after HCQ use is stopped[20]. Besides retinopathy, the other ocular side effects, which tend to be benign or infrequent with low doses of HCQ, include keratopathy, corneal deposits, punctate/linear corneal opacities, infiltrates, ciliary body deposits, ocular muscular imbalance, lens opacities, papilledema, *etc*[21].

Treatment for HCQ toxicity involves cessation of the drug and monitoring for further progression of retinal damage. The prognosis for patients with bull's eye maculopathy due to HCQ toxicity is variable, with some patients experiencing improvement in visual function after cessation of the drug, while others may experience permanent vision loss[20-22]. In some cases, the damage caused by the drug may be delayed and irreversible, resulting in permanent vision loss. There are currently no Food and Drug Administration-approved treatments for bull's eye maculopathy due to HCQ toxicity [22].

In closing, it is important to remember that HCQ can cause several systemic and ophthalmological side effects, most of which are minor and reversible. The prognosis for patients receiving treatment with HCQ for COVID-19 and associated arthritis is generally good. Further research is needed to evaluate the potential long-term ophthalmological side effects associated with the use of the drug to treat and prevent COVID-19 and associated arthritis. Ophthalmological consider-ations should be evaluated prior to initiating the therapy, including baseline visual acuity, fundus examination, and visual field testing to exclude the presence of underlying preexisting ophthalmic retinal disorders. HCQ should be avoided in patients with ophthalmopathies, including any form of retinopathy. Patients receiving HCQ should be period-ically monitored for potential ophthalmological side effects, including retinopathy, corneal deposits, and papilledema. Immediate termination of HCQ and alternative treatment regimens need to be considered in patients that develop HCQ toxicity.

FOOTNOTES

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Country/Territory of origin: Italy

ORCID number: Marco Zeppieri 0000-0003-0999-5545.

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REFERENCES

1 **Bajpai J**, Pradhan A, Verma AK, Kant S. Use of hydroxychloroquine and azithromycin combination to treat the COVID-19 infection. *World J Exp Med* 2022; **12**: 44-52 [PMID: 35765514 DOI: 10.5493/wjem.v12.i3.44]
2 **Liu J**, Cao R, Xu M, Wang X, Zhang H, Hu H, Li Y, Hu Z, Zhong W, Wang M. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. *Cell Discov* 2020; **6**: 16 [PMID: 32194981 DOI: 10.1038/s41421-020-0156-0]
3 **Sanders JM**, Monogue ML, Jodlowski TZ, Cutrell JB. Pharmacologic Treatments for Coronavirus Disease 2019 (COVID-19): A Review. *JAMA* 2020; **323**: 1824-1836 [PMID: 32282022 DOI: 10.1001/jama.2020.6019]
4 **Swarnakar R**, Roy SS, Yadav SL. Can hydroxychloroquine be used for COVID-19-induced arthritis? A debatable hypothesis. *World J Exp Med* 2022; **12**: 108-110 [PMID: 36560976 DOI: 10.5493/wjem.v12.i6.108]
5 **Bansal P**, Goyal A, Cusick A 4th, Lahan S, Dhaliwal HS, Bhyan P, Bhattad PB, Aslam F, Ranka S, Dalia T, Chhabra L, Sanghavi D, Sonani B, Davis JM 3rd. Hydroxychloroquine: a comprehensive review and its controversial role in coronavirus disease 2019. *Ann Med* 2021; **53**: 117-134 [PMID: 33095083 DOI: 10.1080/07853890.2020.1839959]
6 **Shippey EA**, Wagler VD, Collamer AN. Hydroxychloroquine: An old drug with new relevance. *Cleve Clin J Med* 2018; **85**: 459-467 [PMID: 29883308 DOI: 10.3949/ccjm.85a.17034]
7 **Doyno C**, Sobieraj DM, Baker WL. Toxicity of chloroquine and hydroxychloroquine following therapeutic use or overdose. *Clin Toxicol (Phila)* 2021; **59**: 12-23 [PMID: 32960100 DOI: 10.1080/15563650.2020.1817479]
8 **Tehrani R**, Ostrowski RA, Hariman R, Jay WM. Ocular toxicity of hydroxychloroquine. *Semin Ophthalmol* 2008; **23**: 201-209 [PMID: 18432546 DOI: 10.1080/08820530802049962]
9 **Schrezenmeier E**, Dörner T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. *Nat Rev Rheumatol* 2020; **16**: 155-166 [PMID: 32034323 DOI: 10.1038/s41584-020-0372-x]
10 **Savage DE**, Plotnik R, Wozniak RAF. Short-term, high-dose hydroxychloroquine corneal toxicity. *Am J Ophthalmol Case Rep* 2020; **18**: 100713 [PMID: 32346653 DOI: 10.1016/j.ajoc.2020.100713]
11 **Politei J**, Frabasil J, Durand C, Di Pietrantonio S, Fernandez A, Albertón V, Velasquez Rivas D, Barriales-Villa R, Larrañaga-Moreira J, Schenone AB. Incidental finding of cornea verticillata or lamellar inclusions in kidney biopsy: measurement of lyso-Gb3 in plasma defines between Fabry disease and drug-induced phospholipidosis. *Biochim Biophys Acta Mol Basis Dis* 2021; **1867**: 165985 [PMID: 33022387 DOI: 10.1016/j.bbadis.2020.165985]
12 **Yusuf IH**, Sharma S, Luqmani R, Downes SM. Hydroxychloroquine retinopathy. *Eye (Lond)* 2017; **31**: 828-845 [PMID: 28282061 DOI: 10.1038/eye.2016.298]
13 **Modi YS**, Singh RP. Bull's-Eye Maculopathy Associated with Hydroxychloroquine. *N Engl J Med* 2019; **380**: 1656 [PMID: 31018071 DOI: 10.1056/NEJMicm1412167]
14 **Budzinskaya MV**, Durzhinskaya MH. [Differential diagnosis of hydroxychloroquine-induced retinal damage]. *Vestn Oftalmol* 2020; **136**: 265-271 [PMID: 32880149 DOI: 10.17116/oftalma2020136042265]
15 **Lotery A**, Burdon M. Monitoring for hydroxychloroquine retinopathy. *Eye (Lond)* 2020; **34**: 1301-1302 [PMID: 32055019 DOI: 10.1038/s41433-020-0795-2]
16 **Bennett JL**. Optic Neuritis. *Continuum (Minneap Minn)* 2019; **25**: 1236-1264 [PMID: 31584536 DOI: 10.1212/CON.0000000000000768]
17 **Sayeed SKJB**, Khan AH, Moniruzzaman M, Mahmud R, Rahman MM. Systemic lupus erythematosus with acute ischemic optic neuropathy complicated with neuromyelitis optica: a case report. *J Med Case Rep* 2023; **17**: 21 [PMID: 36681831 DOI: 10.1186/s13256-022-03734-8]
18 **Marmor MF**. Hydroxychloroquine at the recommended dose (< or = 6.5 mg/kg/day) is safe for the retina in patients with rheumatoid arthritis and systemic lupus erythematosus. *Clin Exp Rheumatol* 2004; **22**: 143-144 [PMID: 15083878]
19 **Ding HJ**, Denniston AK, Rao VK, Gordon C. Hydroxychloroquine-related retinal toxicity. *Rheumatology (Oxford)* 2016; **55**: 957-967 [PMID: 26428520 DOI: 10.1093/rheumatology/kev357]
20 **Au SCL**. Hydroxychloroquine retinal toxicity: The bull's eye in the human eye. *Vis J Emerg Med* 2020; **21**: 100818 [PMID: 32835115 DOI: 10.1016/j.visj.2020.100818]
21 **Yam JC**, Kwok AK. Ocular toxicity of hydroxychloroquine. *Hong Kong Med J* 2006; **12**: 294-304 [PMID: 16912357]
22 **Jain S**, Jain NG. Delayed progression of bull's eye maculopathy. *BMJ Case Rep* 2017; **2017** [PMID: 28596207 DOI: 10.1136/bcr-2017-220598]

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LETTER TO THE EDITOR

Update on hydroxychloroquine use in pregnancy

Wassan Nori, Nabeeha Najatee Akram, Raid M Al-Ani

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Wassan Nori, Department of Obstetrics and Gynecology, Mustansiriyah University, Baghdad 10052, Iraq

Nabeeha Najatee Akram, Department of Pediatrics, Mustansiriyah University, Baghdad 10052, Iraq

Raid M Al-Ani, Department of Surgery/Otolaryngology, University of Anbar, Anbar 31001, Iraq

Corresponding author: Wassan Nori, PhD, Academic Editor, Academic Research, Senior Researcher, Department of Obstetrics and Gynecology, Mustansiriyah University, No. 58 Al-amin Street, Baghdad 10052, Iraq. dr.wassan76@uomustansiriyah.edu.iq

Abstract

It is well-known that hydroxychloroquine (HCQ) treats malaria, systemic lupus erythematosus, and rheumatoid arthritis in women for its immunomodulatory and anti-inflammatory action. Additionally, HCQ was used in cases with refractory antiphospholipid syndrome. HCQ safety was reinforced in pregnant women owing to insignificant reports of adverse pregnancy outcomes and major congenital malformation. Recently, HCQ was tested in cases with chronic placental inflammation with a promising result of increased life birth; however, its benefit needs further validation. We aimed to highlight the recent updates for HCQ use in various conditions in pregnancy.

Key Words: Pregnancy; Hydroxychloroquine; Preeclampsia; Antiphospholipid syndrome; Chronic placental inflammation; COVID-19

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Core Tip: The immunomodulatory, anti-inflammatory, and anti-thrombotic activity of hydroxychloroquine (HCQ), an anti-malarial drug, made it recommendable for rheumatoid arthritis and systemic lupus erythematosus. HCQ was also implemented in refractory antiphospholipid syndrome showing a successful outcome. Recent evidence supports the benefits of its use to outweigh the risk during pregnancy as it reduces the disease activity and the associated adverse pregnancy outcome. Chronic placental inflammation is another condition for which HCQ proved to be helpful. Further investigations are required to verify HCQ's efficacy in chronic placental inflammation as well as its action in reducing the severity of coronavirus disease 2019 in pregnant women.

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TO THE EDITOR

With interest, we read the Bajpai *et al*[1] study published in the *World Journal of Experimental Medicine* (issue 3, volume 12, 2022) that discussed the role of hydroxychloroquine (HCQ) in treating high-risk groups with coronavirus disease 2019 (COVID-19). Indeed, HCQ gained much interest during the current pandemic owing to its anti-inflammatory and immunomodulatory effects[1].

HCQ had an update regarding its use among pregnant, first in autoimmune diseases and its safety profile-second, its therapeutic role in cases with chronic placental inflammation. Finally, we discuss its potential use in pregnant with COVID-19, which is worth mentioning and was not discussed by Bajpai *et al*'s study[1].

HCQ was already used for treating women suffering from malaria, systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA)[2]. In 2020, the American College for Managing SLE, RA for women of reproductive age, advised that those cases should receive HCQ before and throughout pregnancy[3]. In addition, pregnant women with refractory antiphospholipid syndrome may consider HCQ in addition to standard treatment (aspirin and low molecular weight heparin)[4].

HCQ was used as an adjunctive therapy for cases with refractory antiphospholipid syndrome[5]. It is a beneficial role proposed to be mediated *via* anti-thrombotic, antiplatelet, and immunomodulatory properties[6]. Others suggested that HCQ reduces endothelial dysfunction and improves vascular elasticity, thus improving blood flow[7].

Bérard *et al*'s study addressed pregnancy outcomes related to HCQ use in a cohort study that recruited 233748 pregnant women[8]. Interestingly the study showed that HCQ had a good safety profile. There was no increased risk of preterm labor among drug users; the adjusted odd ratio was 1.39, with respective 95% confidence interval (CI): 0.83 to 2.3. As for the low birth weight, the adjusted odd ratio was 1.12, 95%CI: 0.59 to 2.07. Finally, the adjusted odd ratio for major congenital malformation was 1.02, 95%CI: 0.68 to 1.53[8].

Another study confirmed no substantial rise in significant congenital malformations in newborns exposed to HCQ during the first trimester of pregnancy[9]. In line with earlier work[10,11]. These results reinforce that therapy advantage during pregnancy is likely to exceed the risks for the majority of patients with rheumatic disease.

Ye *et al*[12] discussed that HCQ application might alleviate the risk of high lupus activity during pregnancy and the incidence of preeclampsia. However, in their meta-analysis, Hu *et al*[13] found no value of HCQs in reducing preeclampsia in antiphospholipid syndrome. Moreover, HCQ had no value in reducing fetal growth defects in SLE and/or antiphospholipid cases.

The promising results observed with the use of HCQ to treat autoimmunity in pregnancy have laid the foundation for its use in chronic placental inflammation, a condition characterized by the disruption of healthy placental tissue. They can only be confirmed by a post-delivery histopathological examination[14]. Chronic placental inflammation has been linked to severe complications of pregnancy, such as fetal growth restriction, premature labor, and miscarriage[15].

Brady *et al*'s study examined the value of adding HCQ to pregnant women with a positive history of chronic placental inflammation, showing a decrease in disease severity and a trend for a higher live birth rate[16]. There are currently no prospective, informatively constructed, controlled trials on the efficacy of HCQs in these settings, which emphasizes the need for such work. Since some forms of chronic placental inflammation are recurrent, determining the cause is crucial for future pregnancies care.

The use of HCQ in COVID-19 cases will depend upon if the ongoing clinical trials demonstrate significant benefits of HCQ in reducing the incidence or severity of COVID-19[9,17,18]. Even though initial trials utilizing HCQ to treat COVID-19 failed to demonstrate efficacy, pre-exposure preventative trials are yet to be reported[9].

In conclusion, HCQ has demonstrated efficacy in mitigating the activity of autoimmune diseases and some of their adverse pregnancy outcomes while maintaining a favorable safety profile. HCQ has emerged as a potential therapeutic option for cases with chronic placental inflammation, as it enhances live birth rates while decreasing the severity of the associated disease. Nevertheless, the efficacy and safety of HCQ in pregnant individuals with COVID-19 have not been thoroughly assessed. Further research is needed to unveil more applications in practice.

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FOOTNOTES

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Country/Territory of origin: Iraq

ORCID number: Wassan Nori 0000-0002-8749-2444; Nabeeha Najatee Akram 0000-0001-8964-8943; Raid M Al-Ani 0000-0003-4263-9630.

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REFERENCES

1 **Bajpai J**, Pradhan A, Verma AK, Kant S. Use of hydroxychloroquine and azithromycin combination to treat the COVID-19 infection. *World J Exp Med* 2022; **12**: 44-52 [PMID: 35765514 DOI: 10.5493/wjem.v12.i3.44]
2 **Clowse MEB**, Eudy AM, Balevic S, Sanders-Schmidler G, Kosinski A, Fischer-Betz R, Gladman DD, Molad Y, Nalli C, Mokbel A, Tincani A, Urowitz M, Bay C, van Noord M, Petri M. Hydroxychloroquine in the pregnancies of women with lupus: a meta-analysis of individual participant data. *Lupus Sci Med* 2022; **9** [PMID: 35318256 DOI: 10.1136/lupus-2021-000651]
3 **Sammaritano LR**, Bermas BL, Chakravarty EE, Chambers C, Clowse MEB, Lockshin MD, Marder W, Guyatt G, Branch DW, Buyon J, Christopher-Stine L, Crow-Hercher R, Cush J, Druzin M, Kavanaugh A, Laskin CA, Plante L, Salmon J, Simard J, Somers EC, Steen V, Tedeschi SK, Vinet E, White CW, Yazdany J, Barbhaiya M, Bettendorf B, Eudy A, Jayatilleke A, Shah AA, Sullivan N, Tarter LL, Birru Talabi M, Turgunbaev M, Turner A, D'Anci KE. 2020 American College of Rheumatology Guideline for the Management of Reproductive Health in Rheumatic and Musculoskeletal Diseases. *Arthritis Rheumatol* 2020; **72**: 529-556 [PMID: 32090480 DOI: 10.1002/art.41191]
4 **Nori W**, Shallal F, Zghair MAG. Aspirin effect on Mid luteal Phase Doppler Indices in Patients with Recurrent Pregnancy Loss. *Int J Pharm Res* 2020; **12**: 2929-2934 [DOI: 10.31838/ijpr/2020.12.03.413]
5 **Saraiva-Mangolin S**, Vaz CO, Ruiz T, Mazetto BM, Orsi FA. Use of hydroxychloroquine to control immune response and hypercoagulability in patients with primary antiphospholipid syndrome. *Eur J Intern Med* 2021; **90**: 114-115 [PMID: 34099368 DOI: 10.1016/j.ejim.2021.05.025]
6 **Urbanski G**, Caillon A, Poli C, Kauffenstein G, Begorre MA, Loufrani L, Henrion D, Belizna C. Hydroxychloroquine partially prevents endothelial dysfunction induced by anti-beta-2-GPI antibodies in an *in vivo* mouse model of antiphospholipid syndrome. *PLoS One* 2018; **13**: e0206814 [PMID: 30399161 DOI: 10.1371/journal.pone.0206814]
7 **Dong Y**, Lu Y, Xia Y, Wang X. Effect of hydroxychloroquine on antiphospholipid antibodies-inhibited endometrial angiogenesis. *J Matern Fetal Neonatal Med* 2022; **35**: 7084-7092 [PMID: 34182874 DOI: 10.1080/14767058.2021.1943656]
8 **Bérard A**, Sheehy O, Zhao JP, Vinet E, Quach C, Bernatsky S. Chloroquine and Hydroxychloroquine Use During Pregnancy and the Risk of Adverse Pregnancy Outcomes Using Real-World Evidence. *Front Pharmacol* 2021; **12**: 722511 [PMID: 34408654 DOI: 10.3389/fphar.2021.722511]
9 **Huybrechts KF**, Bateman BT, Zhu Y, Straub L, Mogun H, Kim SC, Desai RJ, Hernandez-Diaz S. Hydroxychloroquine early in pregnancy and risk of birth defects. *Am J Obstet Gynecol* 2021; **224**: 290.e1-290.e22 [PMID: 32961123 DOI: 10.1016/j.ajog.2020.09.007]
10 **Liu J**, Zhang L, Tian Y, Wan S, Hu M, Song S, Zhang M, Zhou Q, Xia Y, Wang X. Protection by hydroxychloroquine prevents placental injury in obstetric antiphospholipid syndrome. *J Cell Mol Med* 2022; **26**: 4357-4370 [PMID: 35770338 DOI: 10.1111/jcmm.17459]
11 **Do SC**, Rizk NM, Druzin ML, Simard JF. Does Hydroxychloroquine Protect against Preeclampsia and Preterm Delivery in Systemic Lupus Erythematosus Pregnancies? *Am J Perinatol* 2020; **37**: 873-880 [PMID: 31899930 DOI: 10.1055/s-0039-3402752]
12 **Ye S**, Zhao X, Liu Y, Ma Y, Wang Y, Zhao J. The use of hydroxychloroquine in pregnancy and its effect on perinatal outcomes in a population with autoimmune abnormalities. *Clin Rheumatol* 2023; **42**: 1137-1150 [PMID: 36507975 DOI: 10.1007/s10067-022-06462-y]
13 **Hu Z**, Gao R, Huang W, Wang H, Qin L. Effect of Hydroxychloroquine on Lupus Activity, Preeclampsia and Intrauterine Growth Restriction in Pregnant Women with Systemic Lupus Erythematosus and/or Antiphospholipid Syndrome: A Systematic Review and Meta-Analysis. *J Clin Med* 2023; **12** [PMID: 36675415 DOI: 10.3390/jcm12020485]
14 **Bouariu A**, Gică N, Ciobanu AM, Scutelnicu AM, Popescu MR, Panaitescu AM. The Potential Benefit of Hydroxychloroquine in Chronic Placental Inflammation of Unknown Etiology Associated with Adverse Pregnancy Outcomes. *Healthcare (Basel)* 2022; **10** [PMID: 35052331 DOI: 10.3390/healthcare10010168]
15 **Cornish EF**, McDonnell T, Williams DJ. Chronic Inflammatory Placental Disorders Associated With Recurrent Adverse Pregnancy Outcome. *Front Immunol* 2022; **13**: 825075 [PMID: 35529853 DOI: 10.3389/fimmu.2022.825075]
16 **Brady CA**, Williams C, Batra G, Church E, Tower CL, Crocker IP, Heazell AEP. Immunomodulatory Therapy Reduces the Severity of Placental Lesions in Chronic Histiocytic Intervillositis. *Front Med (Lausanne)* 2021; **8**: 753220 [PMID: 34733868 DOI: 10.3389/fmed.2021.753220]
17 **Choi EY**, Jeong HE, Noh Y, Choi A, Yon DK, Han JY, Sung JH, Choe SA, Shin JY. Neonatal and maternal adverse outcomes and exposure to nonsteroidal anti-inflammatory drugs during early pregnancy in South Korea: A nationwide cohort study. *PLoS Med* 2023; **20**: e1004183 [PMID: 36848338 DOI: 10.1371/journal.pmed.1004183]
18 **Farhan FS**, Nori W, Al Kadir ITA, Hameed BH. Can Fetal Heart Lie? Intrapartum CTG Changes in COVID-19 Mothers. *J Obstet Gynaecol India* 2022; **72**: 479-484 [PMID: 35634476 DOI: 10.1007/s13224-022-01663-6]



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Editor-in-Chief of *World Journal of Experimental Medicine*, Jian Wu, MD, PhD, Academic Editor, Associate Professor, Doctor, Department of Clinical Laboratory, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Gusu School, Nanjing Medical University, Suzhou 215008, Jiangsu Province, China. wujianglinxing@163.com

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ORIGINAL ARTICLE

Case Control Study

Altered expression of miR-125a and dysregulated cytokines in systemic lupus erythematosus: Unveiling diagnostic and prognostic markers

Tagreed Qassim Alsbihawi, Mojtaba Zare Ebrahimabad, Fakhri Sadat Seyedhosseini, Homa Davoodi, Nafiseh Abdolahi, Alireza Nazari, Saeed Mohammadi, Yaghoub Yazdani

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Tagreed Qassim Alsbihawi, Department of Immunology, Faculty of Medicine, Golestan University of Medical Sciences, Gorgan 49341-74515, Iran

Mojtaba Zare Ebrahimabad, Metabolic Disorders Research Center, Golestan University of Medical Sciences, Gorgan 49341-74515, Iran

Fakhri Sadat Seyedhosseini, Yaghoub Yazdani, Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan 49341-74515, Iran

Homa Davoodi, Department of Immunology, Golestan University of Medical Sciences, Gorgan 4934174515, Iran

Nafiseh Abdolahi, Golestan Rheumatology Research Center, Golestan University of Medical Sciences, Gorgan 49341-74515, Iran

Alireza Nazari, Department of Surgery, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan 7717933777, Iran

Saeed Mohammadi, Golestan Research Center of Gastroenterology and Hepatology, Golestan University of Medical Sciences, Gorgan 49341-74515, Iran

Corresponding author: Saeed Mohammadi, PhD, Assistant Professor, Golestan Research Center of Gastroenterology and Hepatology, Golestan University of Medical Sciences, R9Q4+242, Gorgan 49341-74515, Iran. s.mohammadi@goums.ac.ir

Abstract

BACKGROUND

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder impacting multiple organs, influenced by genetic factors, especially those related to the immune system. However, there is a need for new biomarkers in SLE. MicroRNA-125a (miR-125a) levels are decreased in T cells, B cells, and dendritic cells of SLE patients. MiR-125a plays a regulatory role in controlling the levels of tumor necrosis factor-alpha (TNF-α) and interleukin 12 (IL-12), which are crucial pro-inflammatory cytokines in SLE pathogenesis.

AIM
To assess the levels of miR-125a, IL-12, and TNF-α in SLE patients' plasma, evaluating their diagnostic and prognostic value.

METHODS
The study included 100 healthy individuals, 50 newly diagnosed (ND), and 50 SLE patients undergoing treatment. The patients were monitored for a duration of 24 wk to observe and record instances of relapses. MiR-125a expression was measured using real-time reverse transcription polymerase chain reaction, while ELISA kits were used to assess IL-12 and TNF-α production.

RESULTS
The results showed significantly reduced miR-125a expression in SLE patients compared to healthy individuals, with the lowest levels in ND patients. TNF-α and IL-12 expression levels were significantly elevated in SLE patients, especially in the early stages of the disease. Receiver operating characteristic curve analyses, and Cox-Mantel Log-rank tests indicated miR-125a, TNF-α, and IL-12 as proper diagnostic biomarkers for SLE. A negative correlation was found between plasma miR-125a expression and IL-12/TNF-α levels in SLE patients.

CONCLUSION
Decreased miR-125a levels may be involved in the development of SLE, while elevated levels of IL-12 and TNF-α contribute to immune dysregulation. These findings offer new diagnostic and prognostic markers for SLE. Moreover, the negative correlation observed suggests an interaction between miR-125a, TNF-α, and IL-12. Further research is necessary to uncover the underlying mechanisms that govern these relationships.

Key Words: Systemic lupus erythematosus; microRNA-125a; Interleukin-12; Tumor necrosis factor alpha; Biomarker

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Core Tip: The aim of this study was to investigate the levels of microRNA-125a (miR-125a), interleukin 12 (IL-12), and tumor necrosis factor-alpha (TNF-α) in the plasma of systemic lupus erythematosus (SLE) patients, and assess the diagnostic and prognostic value of these biomarkers in SLE. The study included healthy individuals, newly diagnosed SLE patients, and SLE patients undergoing treatment. The results revealed decreased levels of miR-125a in SLE patients, particularly in newly diagnosed cases. On the other hand, elevated levels of IL-12 and TNF-α were observed in SLE patients, especially in the early stages of the disease. The study also identified miR-125a, TNF-α, and IL-12 as potential diagnostic biomarkers for SLE. The negative correlation observed between miR-125a and IL-12/TNF-α suggests an interaction between these factors. These findings provide insights into new diagnostic and prognostic markers for SLE, highlighting the importance of immune dysregulation in the disease.

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INTRODUCTION

Systemic lupus erythematosus (SLE) involves an overactive immune system causing organ damage and posing significant health risks[1]. SLE significantly reduces quality of life, increases susceptibility to premature death, and imposes substantial financial burdens[2]. Both genetic and environmental factors contribute to the development of SLE, leading to the production of autoantibodies that attack and harm the body's own tissues and organs[3,4]. Epigenetic changes like deoxyribonucleic acid methylation, histone modifications, non-coding RNAs, and chromatin remodeling are associated with SLE development[5,6].

MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression and play a significant role in various biological processes, impacting disease development and progression[7,8]. miRNAs are crucial in regulating immune function and their imbalance is linked to autoimmunity in SLE. Specific miRNAs like miR-146a, miR-155, miR-148a, miR-21, and miR-125a are implicated in SLE[6,9,10]. The imbalance of these miRNAs may contribute to SLE mechanisms by fostering autoreactivity, where the immune system reacts against the body's own cells and tissues[11].

Research suggests reduced miR-125a levels in SLE patients' T cells, B cells, and dendritic cells, leading to increased activation, proliferation, and inflammatory cytokine production[12]. Restoring miR-125a levels shows potential as a treatment target, suppressing abnormal activation[13]. It directly regulates key SLE-related cytokines and serves as a diagnostic and prognostic marker, correlating with disease activity and organ damage[14,15]. Adjusting miR-125a

expression in animal models has shown promise in reducing autoantibody production and improving outcomes[16], although further research is crucial for human application and personalized treatment strategies[11].

Imbalanced and dysfunctional cytokine production and signaling significantly contribute to SLE onset. Dysregulation of cytokines like interleukin (IL)-6, interferon alpha (IFN-α), tumour necrosis factor alpha (TNF-α), IL-17, and IL-12 is linked to SLE development and progression[17]. IL-12, essential in Th1 cell differentiation and immune responses, is linked to SLE severity and lupus nephritis, exerting both pro-inflammatory and immune-regulatory effects. Therapeutic targeting of IL-12 in SLE yields mixed results, reflecting disease complexity and diverse signaling pathways[18-24]. TNF-α, a pro-inflammatory cytokine, exhibits disrupted regulation in SLE, promoting abnormal immune function and autoantibody production. Its role in inflammation includes immune cell recruitment, cytokine and chemokine production, and endothelial cell activation, potentially leading to tissue damage and fibrosis in affected organs. While TNF-α inhibitors hold promise for SLE therapy, identifying responsive patient subsets and optimizing treatment strategies remain essential for improving outcomes[25-29].

Common molecular biomarkers for diagnosing and monitoring SLE include antinuclear antibodies, anti-dsDNA antibodies, complement levels, C-reactive protein, erythrocyte sedimentation rate, IFN-α, and B-cell activating factor. Yet, these markers have limitations such as lack of specificity, sensitivity, and predictability, high costs, and invasive testing, prompting the search for more reliable alternatives[30,31]. MiR-125a regulates TNF-α and IL-12 by targeting their mRNA and influencing related signaling molecules like NF-κB, mitogen activated protein kinases, and signal transducer and activator of transcription proteins. Given the association of TNF-α and IL-12 dysregulation with inflammation, miR-125a holds potential as a therapeutic target. Adjusting miR-125a expression could restore cytokine balance and mitigate inflammation in conditions like rheumatoid arthritis and inflammatory bowel disease, although challenges persist in understanding its regulation[32-35].

To the best of our knowledge, no study has comprehensively examined the expressions of miR-125a, IL-12, and TNF-α in SLE patients collectively and evaluated their diagnostic and prognostic significance. Hence, the aim of this study was to analyze the levels of miR-125a, TNF-α, and IL-12 in the plasma of both SLE patients and healthy individuals, explore their potential correlations, and investigate their usefulness as diagnostic and prognostic biomarkers for SLE.

MATERIALS AND METHODS

Study participants and samplings

A total of 200 participants were enrolled in this study, including 50 newly diagnosed (ND) and 50 under-treatment (UT) SLE patients from the Rheumatology Clinic at Sayyad Shirazi Hospital in Gorgan. Additionally, 100 healthy individuals were included as controls. SLE cases were diagnosed based on the ACR criteria and UT group were receiving standard treatment for SLE without any immunomodulatory or immunosuppressive therapies that could impact the variables under investigation. The control group consisted of age, sex, and ethnicity-matched individuals with no history of autoimmune diseases. All participants were within the age range of 18-65 years and were excluded if they had other autoimmune diseases, active infections, or were pregnant. Under-treatment cases had a minimum disease duration of 6 mo, and their disease activity was recorded at the time of serum collection to minimize the influence of disease activity on the variables of interest. The study received ethical approval from the Committee of Ethics at Golestan University of Medical Sciences (GoUMS), Gorgan, Iran, and adhered to the principles of the Declaration of Helsinki. Informed consent was obtained from all participants prior to their inclusion. Blood samples (5 per participant) were collected and sent to the Research Central Laboratory at GoUMS. Plasma was isolated from the whole blood through centrifugation and stored at -80°C to ensure sample integrity and prevent contamination. Table 1 presents the levels of miR-125a, IL-12, and TNF-α in relation to various clinical characteristics of SLE patients.

ELISA cytokine assay

The expression of cytokines was examined using commercially available ELISA kits from ZellBio (ZellBio GmbH, Germany; IL-12 Cat.NO. RK00072-96; TNF-α Cat.NO. RK00030-96). We strictly adhered to the manufacturer's instructions provided in the kit datasheet to ensure precise and reliable results. The optical density values from the samples and standards were measured using a StatFax 3300 ELISA reader (Awareness Technology, Inc., United States)[36]. Non-linear regression analysis was employed to generate standard curves and calculate the concentrations of IL-12, and TNF-α in each sample, measured in picograms per milliliter (pg/mL) for IL-12 and pg/dL for TNF- α[37].

RNA isolation, cDNA synthesis and real time reverse transcription polymerase chain reaction

Total RNA was extracted from the plasma samples using TRIzol reagent (Invitrogen, United States) following a previously described protocol[38]. The RNA concentration and purity were measured using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, United States). The RNA was either stored at -80°C or used immediately for subsequent applications, ensuring the use of RNase-free reagents and equipment throughout the process. For cDNA synthesis, the Sinnaclon First Strand cDNA Synthesis Kit (Cinnagen, Iran; Cat. NO. RT5201) was employed. To convert mature miRNA molecules into cDNA for amplification and quantification through quantitative polymerase chain reaction (qPCR), the stem-loop method was utilized, along with a specific stem-loop primer and a common reverse primer (as listed in Table 2). The expression levels of miRNA were quantitatively analyzed using Sina Green HS-qPCR Mix (Cinnagen, Iran; Cat. NO. MM2042) along with specific primers. The qPCR reactions were conducted on a Step One Plus cycler (Thermo Fisher Scientific, Iran). In this study, the cycle threshold (Ct) values of miR-125a plasma expression levels were normalized using the internal control U6 (small nuclear RNA U6). The 2-dCt method, a commonly used

Table 1 The association of microRNA-125a, tumor necrosis factor-alpha, and interleukin 12 with major clinical symptoms of systemic lupus erythematosus patients

Characteristics		miR-125a		TNF-α		IL-12	
Lupus nephritis	Yes	0.199 ± 0.07	*P* = 0.576	53.42 ± 14.44	*P* = 0.153	122.37 ± 22.36	*P* = 0.104
	No	0.205 ± 0.09		45.60 ± 12.13		108.34 ± 29.87	
Malar rash	Yes	0.180 ± 0.06	***P* = 0.017**	54.60 ± 12.44	*P* = 0.360	128.45 ± 20.28	*P* = 0.065
	No	0.216 ± 0.09		43.37 ± 11.51		102.42 ± 28.94	
Hair loss	Yes	0.188 ± 0.06	***P* = 0.022**	52.63 ± 13.08	*P* = 0.401	126.87 ± 20.62	***P* = 0.049**
	No	0.211 ± 0.09		44.34 ± 11.98		103.19 ± 29.48	
SLEDAI	≤ 4	0.202 ± 0.08	***P* = 0.005**	44.00 ± 14.71	***P* = 0.001**	102.43 ± 23.38	***P* = 0.000**
	5-12	0.280 ± 0.08		30.49 ± 12.59		69.54 ± 32.43	
	≥ 12	0.199 ± 0.09		49.11 ± 13.51		113.26 ± 28.54	

Table 2 List of primers for microRNA-125a and U6 internal control

Primer	Sequence (5'>3')
miR-125a	**F:** GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCGT
	R: GTCGTATCCAGTGCAGGGTCCGAGGTGCCGAGGATTTCCACCACCTG
U6	**F:** GCTTCGGCAGCACATATACTAAAAT
	R: CGCTTCACGAATTTGCGTGTCAT

approach for normalizing gene and miRNA expression levels, was utilized for data analysis[39].

Statistical analysis

Statistical analyses were performed using SPSS 22.0 (IBM Corporation, United States) and Prism 8.0 (GraphPad Software Inc, United States). The Shapiro-Wilk test was used to assess the normality of the data, and parametric or non-parametric tests were chosen accordingly. Independent Samples *t*-test or Mann-Whitney U test was employed for comparisons between two groups, while one-way ANOVA with Tukey's post-test or Kruskal-Wallis with Dunn-Bonferroni post-test was used for comparisons involving more than two groups. Pearson/Spearman tests were utilized for correlation analyses based on the distribution of the data. Receiver operating characteristic (ROC) curve analyses were conducted to evaluate the diagnostic utility of each variable, and logistic regression was employed for combined ROC curve analysis and prediction of variable performance. The Mantel-Haenszel (Mantel-Cox) log-rank test was utilized to assess the prognostic value of variables in predicting flare occurrence after a 24-wk follow-up. All experiments were performed in triplicates. The significance level for all statistical tests was set at 0.05, with a 95% confidence interval and a test power of 80%.

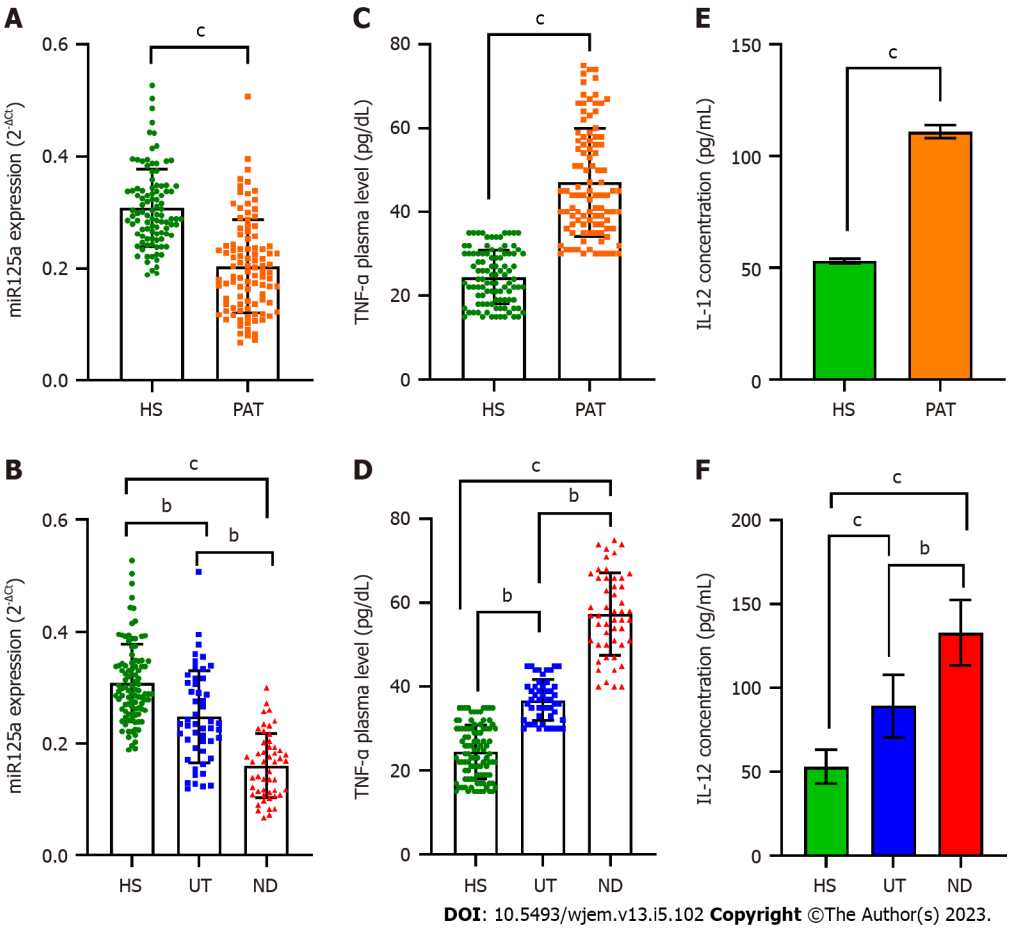
RESULTS

Expression levels of miR-125a, TNF-α, and IL-12 in SLE patients

This study analyzed the expression levels of miR125a, TNF-α, and IL-12 in SLE patients and normal subjects. The results indicated that the expression level of miR-125a was significantly decreased in SLE patients compared to normal subjects (*P* value < 0.0001) (Figure 1A). However, it was shown that miR-125a expression was lowest in ND SLE patients compared to those UT (*P* value < 0.01) (Figure 1B). TNF-α was significantly elevated in SLE patients compared to normal subjects (*P* value < 0.0001) (Figure 1C). The expression level of TNF-α was compared among SLE patients with different disease states (under treatment and newly diagnosed). The results showed that TNF-α expression was highest in ND SLE patients compared to UT patients (*P* value < 0.01) (Figure 1D). Independent samples t-test indicated that the expression level of IL-12 was significantly elevated in SLE patients compared to normal subjects (*P* value < 0.0001) (Figure 1E). The expression level of IL-12 was highest in ND SLE patients compared to those with longer disease durations (*P* value < 0.01) (Figure 1F).

The diagnostic utilities of miR-125a, TNF-α and IL-12

ROC curve analysis was conducted to evaluate the diagnostic utility of miR-125a, TNF-α and IL-12 to distinguish SLE patients from normal subjects, and ND SLE patients from UT. The area under the curve (AUC) for the expression of miR-



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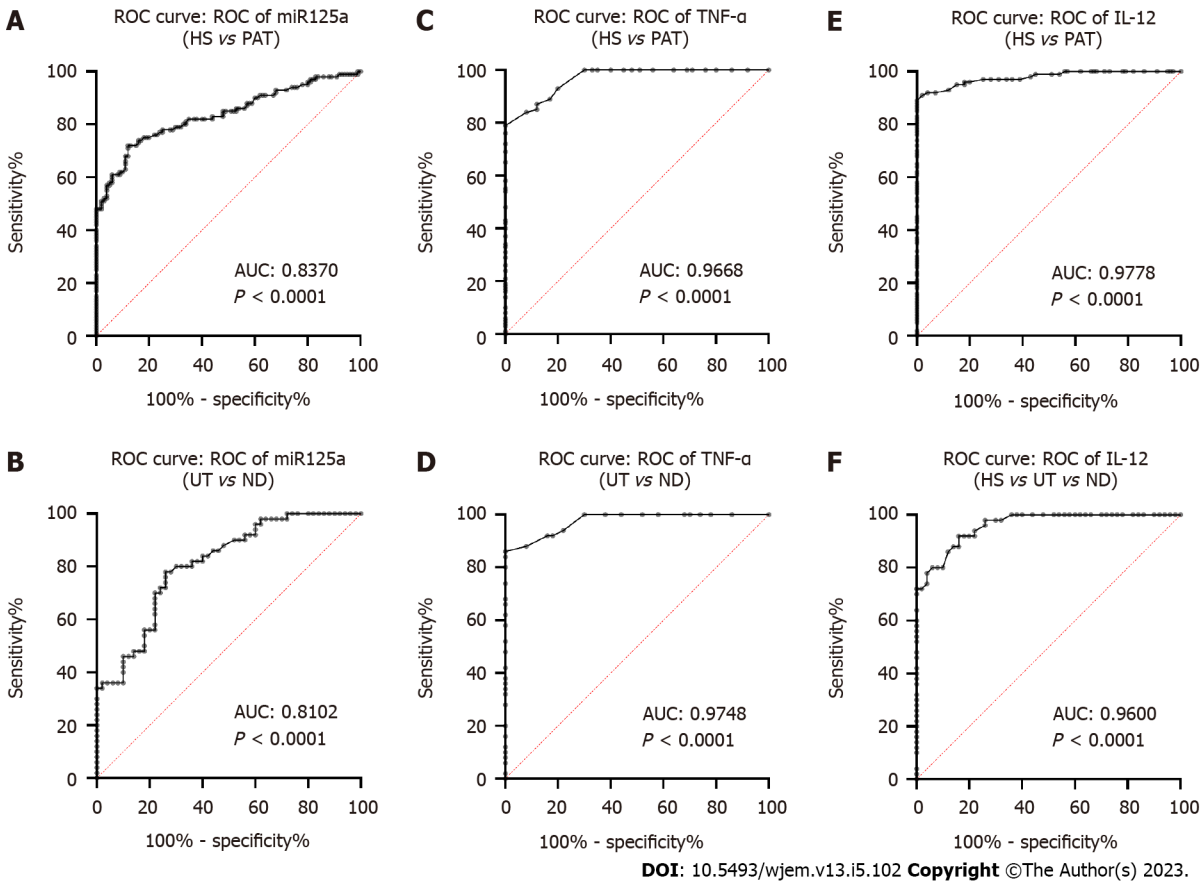
Figure 1 The expression levels of microRNA-125a, tumor necrosis factor-alpha, and interleukin 12 in systemic lupus erythematosus patients and normal subjects. This study examined the expression levels of microRNA-125a (miR-125a), tumor necrosis factor-alpha (TNF-α), and interleukin 12 (IL-12) in systemic lupus erythematosus (SLE) patients and normal subjects. A: The findings revealed that miR-125a expression was significantly lower in SLE patients compared to normal subjects; B: With the lowest levels observed in newly diagnosed patients; C: TNF-α expression was higher in SLE patients compared to normal subjects; D: Its levels were highest in newly diagnosed patients; E: Similarly, the expression of IL-12 was significantly elevated in SLE patients compared to normal subjects; F: It was highest in newly diagnosed patients. The Independent Samples *t*-test or Mann-Whitney U test were employed for comparing two groups, while one-way ANOVA with Tukey's post-test or Kruskal-Wallis with Dunn-Bonferroni post-test were used for comparing more than two groups. The error bars represent means ± SD. Significance levels are denoted as $^{b}P < 0.01$ and $^{c}P < 0.0001$. HS: Humic subjects; PAT: Patients; UT: Under-treatment; ND: Newly diagnosed.

125a [humic subjects (HS) *vs* Patients (PAT)] (Figure 2A) was 0.8370 (95%CI: 0.7803 to 0.8936; $P < 0.0001$). The cut-off point was set at the fold change level of 0.2365 with the sensitivity of 72.00% (95%CI: 62.51% to 79.86%), the specificity of 88.00% (95%CI: 80.19% to 93.00%), and likelihood ratio (LR) of 6.0. The calculated AUC for miR-125a (ND *vs* UT) (Figure 2B) was 0.8102 (95%CI: 0.7279 to 0.8925; $P < 0.0001$). The cut-off value was set at the FC level of 0.1849 with the sensitivity of 70.0% (95%CI: 56.25% to 80.90%), the specificity of 78.0% (95%CI: 64.76% to 87.25%), and LR of 3.182.

The AUC for the expression of TNF-α (HS *vs* PAT) (Figure 2C) was 0.9668 (95%CI: 0.9476 to 0.9860; $P < 0.0001$). The cut-off point was set at the level of 34.50 with the sensitivity of 84.00% (95%CI: 75.58% to 89.90%), the specificity of 92.00% (95%CI: 85.00% to 95.89%), and LR of 10.50. Similarly, the calculated AUC for TNF-α (ND *vs* UT) (Figure 2D) was 0.9748 (95%CI: 0.9513 to 0.9983; $P < 0.0001$). The cut-off value was set at the level of 44.50 with the sensitivity of 88.00% (95%CI: 76.20% to 94.38%), the specificity of 92.00% (95%CI: 81.16% to 96.85%), and LR of 11.00. The AUC for the expression of IL-12 (HS *vs* PAT) (Figure 2E) was 0.9778 (95%CI: 0.9599 to 0.9957; $P < 0.0001$). The cut-off point was set at the level of 69.50 pg/mL with the sensitivity of 91.00% (95%CI: 83.77% to 95.19%), the specificity of 98.00% (95%CI: 93.00% to 99.64%), and LR of 45.5. Similarly, the calculated AUC for IL-12 (ND *vs* UT) (Figure 2F) was 0.9600 (95%CI: 0.9289 to 0.9911; $P < 0.0001$). The cut-off value was set at the level of 119.50 pg/mL with the sensitivity of 72% (95%CI: 58.33% to 82.53%), the specificity of 98% (95%CI: 89.50% to 99.90%), and LR of 36.0.

The correlations of miR-125a with TNF-α and IL-12

We examined the relationship between plasma levels of miR-125a and IL-12 as well as miR-125a and TNF-α. The results of a Pearson correlation analysis revealed a negative correlation between IL-12 and miR-125a ($r = -0.569$, $P < 0.0001$) (Figure 3A). Similarly, a negative correlation was observed between TNF-α and miR-125a ($r = -0.570$, $P < 0.0001$) (Figure 3B).



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Figure 2 Diagnostic utilities of microRNA-125a, tumor necrosis factor-alpha, and interleukin 12 in systemic lupus erythematosus patients. Receiver operating characteristic curve analysis was performed to assess the diagnostic accuracy of microRNA-125a (miR-125a), tumor necrosis factor-alpha (TNF-α), and interleukin 12 (IL-12) in distinguishing systemic lupus erythematosus (SLE) patients from normal subjects and newly diagnosed SLE patients from those under treatment. A: The area under the curve (AUC) values for miR-125a were 0.8370 (95%CI: 0.7803 to 0.8936; $P < 0.0001$) in SLE patients *vs* normal subjects; B: 0.8102 (95%CI: 0.7279 to 0.8925; $P < 0.0001$) in newly diagnosed *vs* under treatment SLE patients; C: For TNF-α, the AUC values were 0.9668 (95%CI: 0.9476 to 0.9860; $P < 0.0001$) in SLE patients *vs* normal subjects; D: 0.9748 (95%CI: 0.9513 to 0.9983; $P < 0.0001$) in newly diagnosed vs. under treatment SLE patients; E: Regarding IL-12, the AUC values were 0.9778 (95%CI: 0.9599 to 0.9957; $P < 0.0001$) in SLE patients *vs* normal subjects; F: 0.9600 (95%CI: 0.9289 to 0.9911; $P < 0.0001$) in newly diagnosed *vs* under treatment SLE patients. HS: Humic subjects; PAT: Patients; UT: Under-treatment; ND: Newly diagnosed.

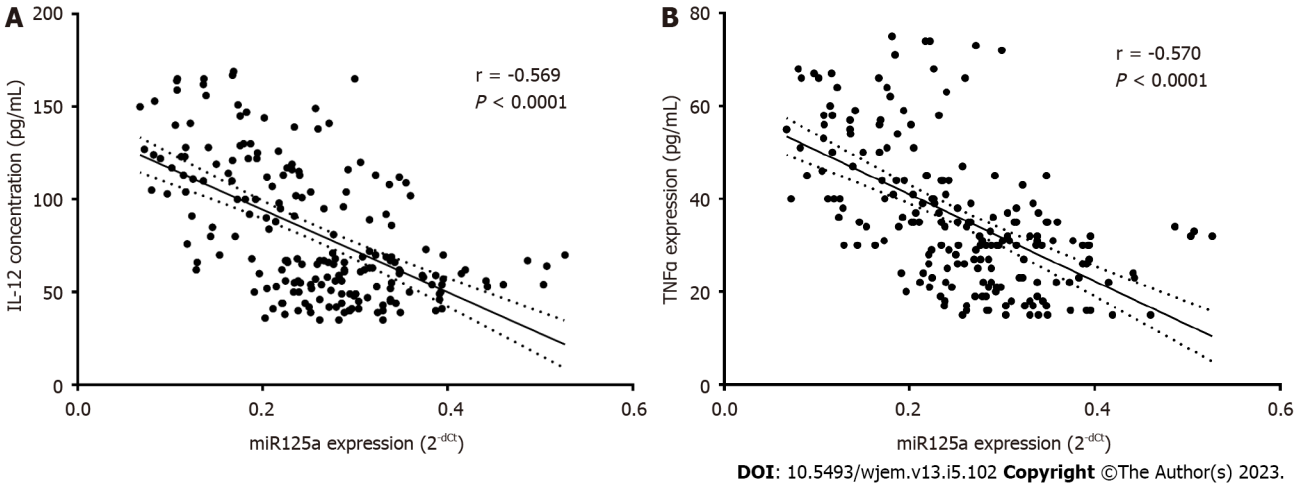
The prognostic utilities of miR-125a, TNF-α and IL-12

The levels of miR-125a, TNF-α, and IL-12 were categorized as low or high based on the optimal cut-off points determined from ROC curve analyses. The predictive ability of these biomarkers for the outcome (Flare) of SLE patients was assessed using the log-rank test. The results showed that miR-125a did not significantly predict the outcome of SLE patients ($P < 0.7151$) (Figure 4A). TNF-α, on the other hand, had a predictive potential for the outcome of SLE patients, but the association was not statistically significant ($P = 0.4828$) (Figure 4B). In contrast, IL-12 demonstrated a significant predictive ability for the outcome of SLE patients ($P = 0.0508$) (Figure 4C) according to the log-rank test.

DISCUSSION

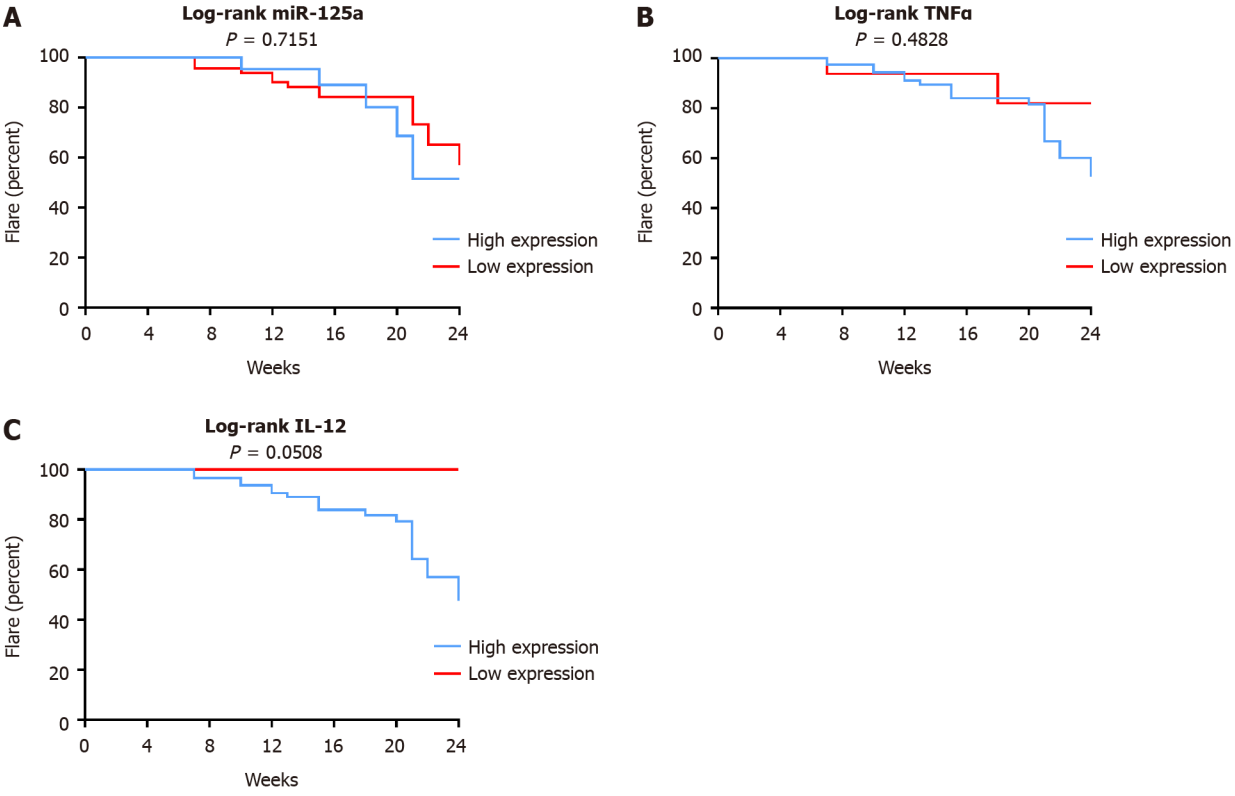
Recent research has focused on exploring the diagnostic and prognostic potential of various biomarkers in SLE. Our study investigated the levels of miR-125a, IL-12, and TNF-α in the plasma of individuals with SLE. The main objective was to evaluate the usefulness of these biomarkers in predicting SLE flares after 24 wk. By analyzing their diagnostic utilities and prognostic power, this study aimed to improve the detection and management of SLE.

Our study found a significant decrease in miR125a expression in SLE patients compared to normal individuals. Furthermore, miR125a expression was observed to be lowest in newly diagnosed SLE patients as compared to those who were already under treatment. These results are consistent with the findings of Zhao *et al*[15], who showed reduced expression of miR-125a and increased expression of its predicted target gene KLF13 in SLE patients. The study by Zhao *et al*[15] had limited samples, while our study had a larger sample size. Furthermore, while Zhao *et al*[15] did not categorize their SLE patients based on disease duration, our study did so and revealed that miR-125a expression is more reduced in newly-diagnosed SLE patients. Consequently, our findings suggest that treatment may have an impact on miR-125a expression levels in SLE patients. A study conducted by Zhang *et al*[40] also aimed to investigate the roles of circRNAs in SLE and their findings were consistent with ours. They utilized microarray analysis, which was verified by qPCR, to



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Figure 3 The correlations of microRNA-125a with tumor necrosis factor-alpha and tumor necrosis factor-alpha. A: The relationship between plasma levels of microRNA-125a (miR-125a) and interleukin 12 was assessed, revealing a significant negative correlation (r = -0.569, P < 0.0001) as demonstrated by Pearson correlation analysis; B: Similarly, the plasma levels of miR-125a and tumor necrosis factor-alpha showed a significant negative correlation (r = -0.570, P < 0.0001) based on the Pearson correlation study.



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Figure 4 The prognostic utilities of microRNA-125a, tumor necrosis factor-alpha, and interleukin 12 to predict flare in systemic lupus erythematosus patients. Based on the optimal cut-off points obtained from receiver operating characteristic curve analyses, the levels of microRNA-125a (miR-125a), tumor necrosis factor-alpha (TNF-α), and interleukin 12 (IL-12) were categorized as low or high. The predictive ability of these biomarkers for the outcome (Flare) in systemic lupus erythematosus (SLE) patients was assessed using the log-rank test. A: The results showed that miR-125a was not significantly predictive of the outcome (P < 0.7151); B: TNF-α showed a potential for predicting the outcome, but the association was not statistically significant (P = 0.4828); C: In contrast, IL-12 demonstrated a significant predictive capability for the outcome in SLE patients (P = 0.0508) based on the log-rank test results.

demonstrate that miR-125a was downregulated in SLE patients as compared to healthy controls, and this reduction was linked to SLE characteristics. However, their sample size was limited (n = 3), which may have affected the generalizability of their results. Our results were consistent with those reported by Nascimento *et al*[41], who observed downregulation of miR-125 in peripheral blood mononuclear cells (PBMCs) of childhood-onset systemic lupus erythematosus (cSLE) patients. However, their study was limited to cSLE patients and PBMC samples, while our investigation focused on adult

females and plasma samples. While our findings regarding reduced miR-125a expression in SLE patients are similar to those reported by Eissa *et al*[42], there is a difference in the focus of our studies. Eissa *et al*[42] evaluated the plasma expression of miR-125a specifically in juvenile SLE patients, whereas our study did not differentiate between adult and juvenile SLE patients. In summary, our study provides additional evidence to support the idea that levels of miR-125a are lower in patients with SLE and decrease further in more severe cases of the disease.

The expression of miR-125a was found to be significantly different between individuals with SLE and those without the disease. To determine whether miR-125a could be a useful diagnostic tool for detecting SLE and distinguishing between newly diagnosed patients and those already undergoing treatment, we analyzed ROC curves. The results showed that miR-125a has an AUC of 0.8370 and 0.8102 for distinguishing SLE patients from normal individuals and newly diagnosed patients from those already under treatment, respectively. This suggests that miR-125a is effective in identifying SLE patients and differentiating them from healthy individuals or those at different stages of treatment. Biomarkers used for diagnosis can be classified based on their AUC values, which determine how accurately they can differentiate between two groups, such as healthy and diseased individuals. An AUC value of 0.9-1.0 indicates an excellent biomarker, meaning that it is highly dependable in identifying the presence or absence of a target condition. A biomarker with an AUC value between 0.8-0.9 is considered good, indicating reasonably accurate diagnostic ability. An AUC value of 0.7-0.8 is classified as fair, meaning that it has moderate diagnostic accuracy but may not be as reliable. Biomarkers with an AUC value below 0.7 should be considered poor and should not be relied upon for diagnoses[43]. Therefore, miR-125a is classified as an effective diagnostic biomarker for SLE. While Zhang *et al*[40] have suggested that miR-125a could serve as a diagnostic biomarker for SLE based on its significant reduction in SLE patients, and several studies have introduced miR-125a as diagnostic biomarkers for malignancies such as Pancreatic Cancer[44], and Cervical Cancer[45], our study is, to the best of our knowledge, the first to propose miR-125a as an effective diagnostic biomarker for both discriminating between SLE patients and healthy subjects, as well as distinguishing under-treatment patients from newly diagnosed ones. In this study, patients were also divided into high and low expression groups based on the suggested miR-125a cut-off point. These individuals were then monitored for 24 wk to determine if they experienced a flare or not. Based on the results of the log-rank test, it was concluded that miR-125a is not a predictor of SLE patient outcomes after 24 wk. It should be noted that, to date, no other research has explored the utility of miR-125a as a prognostic marker for SLE flare outcome. Further research is required to confirm these findings.

Regarding the function of miR-125a in SLE pathogenesis, Zhang and his colleagues suggested that miR-125a may play a role in the development of SLE. They speculated that miR-125a could help maintain self-tolerance by limiting the activity of T cells that promote inflammation, but they found that its expression is lower in T cells from individuals with SLE. Based on these findings, they proposed that increasing levels of miR-125a might be a promising strategy for treating SLE[46]. In their study, Zhao and colleagues discovered that the expression of miR-125a was lower in individuals with SLE. They also observed an increase in KLF13, a gene that miR-125a is predicted to target. When miR-125a was overexpressed, it led to a significant decrease in the expression of RANTES and KLF13. The researchers found that miR-125a inhibited KLF13 expression in a dose-dependent manner using gain- and loss-of-function methods. Additionally, introducing miR-125a into T cells from SLE patients reduced the high levels of RANTES expression. Notably, the expression of miR-125a in T cells increased in a dose- and time-dependent manner[15]. However, further research is needed.

Our study analyzed the expression level of TNF-α in SLE patients and normal subjects. The results showed that the expression level of TNF-α was significantly elevated in SLE patients compared to normal subjects. Additionally, TNF-α expression was highest in newly diagnosed SLE patients compared to those with longer disease durations. ROC curve analysis was used to assess the diagnostic accuracy of TNF-α in distinguishing SLE patients from normal subjects and newly diagnosed SLE patients from those under treatment. The AUC for TNF-α expression was high in both cases, indicating excellent diagnostic utility. However, Log-rank test results revealed that TNF-α was not capable of predicting the outcome (Flare) of SLE patients, after 24 wk. The role of TNF-α in the pathogenesis of SLE is well established, and our findings are supported by several confirmatory studies, further strengthening their significance. According to the results of their research, Idborg and colleagues carried out a study aimed at determining potential biomarkers for identifying disease activity in patients with SLE by evaluating a large group of cytokines and basic laboratory tests. They discovered that TNF-α had the most significant ability to differentiate between SLE patients and healthy individuals, with higher levels detected in SLE patients. The researchers also observed a strong association between TNF-α and measures of disease activity, particularly in the subgroup of patients with nephritis[47]. In research conducted by Ma and colleagues, it was observed that SLE patients had higher levels of plasma TNF-α than healthy individuals, which is consistent with our own results. Additionally, the study found that TNF-α levels were elevated in SLE patients who were experiencing active symptoms compared to those who were not, as well as compared to healthy controls, which agrees with previous findings of increased TNF-α expression in patients who have recently been diagnosed with SLE[48]. In addition to their other findings, Rana and colleagues discovered that the TNF-α gene was highly expressed in most patients with SLE. Furthermore, they observed a strong association between these expression levels and both renal involvement and disease activity as measured by SLE Disease Activity Index (SLEDAI) scores[49]. According to Sabry and colleagues, SLE patients with active hematological disease exhibited elevated levels of TNF-α as compared to those with inactive disease or healthy individuals. Additionally, they found a strong positive correlation between the TNF-α levels and the SLEDAI score. The results of their study indicated that increased levels of TNF-α may contribute to the onset of anemia among Egyptian patients with Lupus Nephritis[50]. According to our research, a study conducted by Sabry *et al*[27] came up with similar results. They found that patients who had active SLE had significantly higher levels of TNF-α compared to those who had inactive SLE or were healthy. They concluded that measuring the levels of TNF-α in the blood could be a useful way to predict disease activity and distinguish between individuals with SLE and those without. The elevated levels of TNF-α and its possible role as a diagnostic marker was also confirmed in other studies by Aringer *et al*[51],

Umare *et al*[52], and Zhu *et al*[53].

Regarding the association between TNF-α and diverse clinical manifestations in SLE, notably, patients with CNS involvement exhibit markedly elevated TNF-α levels in contrast to those without CNS involvement. Furthermore, TNF-α levels are significantly heightened in lupus patients overall, particularly in those with NPLE. Additionally, a substantial TNF-α level increase is observed in patients presenting with multiple focal pattern hypoperfusion, the predominant SPECT pattern in individuals with NPLE[54]. Diffusion tensor imaging (DTI) relies on assessing water diffusion within cellular compartments. In this context, DTI holds the potential to serve as an imaging biomarker for neuropsychiatric systemic lupus erythematosus and a valuable tool for correlation with TNF-α levels[55].

We also analyzed the expression level of IL-12 in SLE patients and normal subjects. The results revealed that the expression level of IL-12 was significantly higher in SLE patients than in normal subjects. Additionally, among SLE patients, those who were newly diagnosed had the highest levels of IL-12 expression compared to those who had been under treatment for longer periods. The diagnostic ability of IL-12 to distinguish SLE patients from normal subjects and newly diagnosed SLE patients from those under treatment was evaluated through ROC curve analysis, suggesting that IL-12 may be a useful diagnostic marker for SLE, particularly in distinguishing between newly diagnosed and under-treated patients. Following the patients for 24 wk, Log-rank test results revealed that IL-12 was capable of predicting the outcome (Flare) of SLE patients. Similarly, the role of IL-12 in SLE pathogenesis is established in previous studies, and there are several studies highlighting the elevation of IL-12 in SLE patients. According to our discoveries, Capper and colleagues demonstrated that the levels of IL-12 were noticeably elevated in individuals with SLE when compared to those who did not have the condition. This difference was observed irrespective of whether the SLE patients were having an active episode of the disease or not[56]. Lauwerys and colleagues also noted similar results, where they observed an increase in IL-12 p40 levels in the blood of SLE patients. They further demonstrated that the administration of immunosuppressive therapy resulted in a significant decrease in serum IL-12 levels, which verified the lower levels of IL-12 found in individuals without SLE in our study[20], supported in the studies conducted by Qiu *et al*[57], and, Uzrail *et al*[58].

Despite the significant role of IL-12 in SLE pathogenesis, few studies assessed its diagnostic and prognostic accuracy for SLE. In a study by Ye *et al*[22] SLE patients had significantly higher plasma levels of IL-12, and the area under the curve (AUC) for IL-12 was 0.756, indicating its potential as biomarkers for SLE diagnosis. Through our study on a larger sample size, we have discovered that IL-12 can serve as an excellent biomarker for SLE. While Ye *et al*[22] identified it as a fair biomarker, our findings indicate its potential as a stronger indicator of the disease. Additionally, our study is the first to evaluate the occurrence of flares in SLE patients after 24 wk of follow-up. Thus, our results provide valuable insights into the long-term management and prognosis of SLE.

While our study yielded promising results regarding the potential diagnostic and prognostic utilities of miR-125a, TNF-α, and IL-12 in SLE patients, we acknowledge that there were limitations to our research. Despite having a larger sample size compared to previous studies, the generalizability of our findings could be further improved by increasing the sample size even more. Furthermore, as our study only assessed the plasma levels of these molecules, it may be worthwhile to explore their expression in different types of cells or tissues to gain a more comprehensive understanding of their role in SLE pathogenesis. Moreover, it is important to note that our study had a cross-sectional design which limits our ability to establish a cause-and-effect relationship between the levels of these molecules and disease progression. Therefore, we suggest conducting a longitudinal cohort study to further investigate the potential causal relationships between miR-125a, TNF-α, and IL-12 levels and SLE progression.

CONCLUSION

Our study has shed light on the potential of miR-125a, TNF-α, and IL-12 as biomarkers for SLE diagnosis and management. We found that the downregulation of miR-125a is an effective diagnostic tool for distinguishing between SLE patients and healthy individuals, as well as newly diagnosed patients from those under treatment. Furthermore, TNF-α and IL-12 levels were elevated in SLE patients, with TNF-α serving as a useful diagnostic marker and IL-12 being both a diagnostic and prognostic marker for SLE flare outcome. These findings could contribute to improved patient outcomes and better management of SLE. However, further research is needed to fully understand the mechanisms by which these biomarkers are involved in SLE pathogenesis and their potential as therapeutic targets.

ARTICLE HIGHLIGHTS

Research background

Systemic lupus erythematosus (SLE) is a long-lasting autoimmune disorder that impacts multiple organs and significantly increases the risk of morbidity and mortality. MicroRNA-125a (miR-125a) levels are decreased in T cells, B cells, and dendritic cells of SLE patients. MiR-125a plays a regulatory role in controlling the levels of tumor necrosis factor-alpha (TNF-α) and interleukin 12 (IL-12), which are crucial pro-inflammatory cytokines in SLE pathogenesis.

Research motivation

Recent research has focused on exploring the diagnostic and prognostic potential of various biomarkers in SLE. Since the levels of miR-125a, TNF-α, and IL-12 are altered in SLE, these molecules could be introduced as novel biomarkers.

Research objectives

The aim of this study was to analyze the levels of miR-125a, TNF-α, and IL-12 in the plasma of both SLE patients and healthy individuals, explore their potential correlations, and investigate their usefulness as diagnostic and prognostic biomarkers for SLE.

Research methods

The study included 100 healthy individuals, 50 newly diagnosed (ND), and 50 SLE patients undergoing treatment. The patients were monitored for a duration of 24 wk to observe and record instances of relapses. MiR-125a expression was measured using real-time reverse transcription polymerase chain reaction, while ELISA kits were used to assess IL-12 and TNF-α production.

Research results

The results showed significantly reduced miR-125a expression in SLE patients compared to healthy individuals, with the lowest levels in ND patients. TNF-α and IL-12 expression levels were significantly elevated in SLE patients, especially in the early stages of the disease. Receiver operating characteristic curve analyses, and Cox-Mantel Log-rank tests indicated miR-125a, PDCD4, and IL-10 as proper diagnostic biomarkers for SLE. A negative correlation was found between plasma miR-125a expression and IL-12/TNF-α levels in SLE patients.

Research conclusions

Decreased miR-125a levels may be involved in the development of SLE, while elevated levels of IL-12 and TNF-α contribute to immune dysregulation. These findings offer new diagnostic and prognostic markers for SLE. Moreover, the negative correlation observed suggests an interaction between miR-125a, TNF-α, and IL-12.

Research perspectives

Further research is necessary to uncover the underlying mechanisms that govern the relationships between miR-125a, TNF-α, and IL-12 in SLE pathogenesis.

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FOOTNOTES

Author contributions: Alsbihawi TQ, Seyedhosseini F, Davoodi H, Mohammadi S, and Yazdani Y contributed to conceptualization (Mohammadi S and Yazdani Y), contributed to methodology; Alsbihawi TQ, Ebrahimabad MZ, and Mohammadi S contributed to software; Alsbihawi TQ, Mohammadi S, and Yazdani Y contributed to validation; Alsbihawi TQ, and Mohammadi S contributed to formal analysis; Alsbihawi TQ, Ebrahimabad MZ, and Mohammadi S contributed to investigation; Alsbihawi TQ, Mohammadi S, and Yazdani Y contributed to resources; Alsbihawi TQ, Mohammadi S, and Yazdani Y contributed to data curation; Alsbihawi TQ, Ebrahimabad MZ, Seyedhosseini FS, Davoodi H, Abdolahi N, Mohammadi S, and Yazdani Y contributed to writing – original draft preparation; Alsbihawi TQ, Ebrahimabad MZ, Seyedhosseini FS, Davoodi H, Abdolahi N, Mohammadi S, and Yazdani Y contributed to writing – review and editing; Mohammadi S and Yazdani Y contributed to supervision; Mohammadi S and Yazdani Y contributed to project administration; Mohammadi S and Yazdani Y contributed to funding acquisition; All authors have read and approved the final version of the manuscript.

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Informed consent statement: All participants provided informed consent prior to their involvement. Utmost confidentiality and privacy were ensured throughout the study, and precautionary measures were implemented to prevent any form of undue influence or coercion.

Conflict-of-interest statement: All the authors declare that there are no conflicts of interest to disclose.

Data sharing statement: The data supporting the findings of this study will be made accessible upon reasonable request by the corresponding author, Dr. Saeed Mohammadi (s.mohammadi@goums.ac.ir).

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Country/Territory of origin: Iran

ORCID number: Homa Davoodi 0000-0002-4198-5411; Nafiseh Abdolahi 0000-0003-1190-5449; Alireza Nazari 0000-0001-8352-793X; Saeed Mohammadi 0000-0001-9895-8468; Yaghoub Yazdani 0000-0003-4005-3521.

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REFERENCES

1 **Dörner T**, Furie R. Novel paradigms in systemic lupus erythematosus. *Lancet* 2019; **393**: 2344-2358 [PMID: 31180031 DOI: 10.1016/S0140-6736(19)30546-X]
2 **Danchenko N**, Satia JA, Anthony MS. Epidemiology of systemic lupus erythematosus: a comparison of worldwide disease burden. *Lupus* 2006; **15**: 308-318 [PMID: 16761508 DOI: 10.1191/0961203306lu2305xx]
3 **Pons-Estel GJ**, Alarcón GS, Scofield L, Reinlib L, Cooper GS. Understanding the epidemiology and progression of systemic lupus erythematosus. *Semin Arthritis Rheum* 2010; **39**: 257-268 [PMID: 19136143 DOI: 10.1016/j.semarthrit.2008.10.007]
4 **Saghaeian Jazi M**, Mohammadi S, Zare Ebrahimabad M, Sedighi S, Abdolahi N, Tabarraei A, Yazdani Y. Genetic variation in CYP1A1 and AHRR genes increase the risk of systemic lupus erythematosus and exacerbate disease severity in smoker patients. *J Biochem Mol Toxicol* 2021; **35**: e22916 [PMID: 34580959 DOI: 10.1002/jbt.22916]
5 **Markopoulou A**, Kyttaris VC. Small molecules in the treatment of systemic lupus erythematosus. *Clin Immunol* 2013; **148**: 359-368 [PMID: 23158694 DOI: 10.1016/j.clim.2012.09.009]
6 **Eghbalpour F**, Aghaei M, Ebrahimi M, Tahsili MR, Golalipour M, Mohammadi S, Yazdani Y. Effect of indole-3-carbinol on transcriptional profiling of wound-healing genes in macrophages of systemic lupus erythematosus patients: an RNA sequencing assay. *Lupus* 2020; **29**: 954-963 [PMID: 32517571 DOI: 10.1177/0961203320929746]
7 **Pasquinelli AE**, Hunter S, Bracht J. MicroRNAs: a developing story. *Curr Opin Genet Dev* 2005; **15**: 200-205 [PMID: 15797203 DOI: 10.1016/j.gde.2005.01.002]
8 **Hammond SM**. An overview of microRNAs. *Adv Drug Deliv Rev* 2015; **87**: 3-14 [PMID: 25979468 DOI: 10.1016/j.addr.2015.05.001]
9 **Ibrahim SA**, Afify AY, Fawzy IO, El-Ekiaby N, Abdelaziz AI. The curious case of miR-155 in SLE. *Expert Rev Mol Med* 2021; **23**: e11 [PMID: 34470679 DOI: 10.1017/erm.2021.11]
10 **Omidi F**, Khoshmirsafa M, Kianmehr N, Faraji F, Delbandi A, Seif F, Shekarabi M. Comparison of circulating miR-148a and miR-126 with autoantibodies as biomarkers of lupus nephritis in patients with SLE. *J Immunoassay Immunochem* 2022; **43**: 634-647 [PMID: 35938736 DOI: 10.1080/15321819.2022.2099225]
11 **Miao CG**, Yang YY, He X, Huang C, Huang Y, Zhang L, Lv XW, Jin Y, Li J. The emerging role of microRNAs in the pathogenesis of systemic lupus erythematosus. *Cell Signal* 2013; **25**: 1828-1836 [PMID: 23707525 DOI: 10.1016/j.cellsig.2013.05.006]
12 **Choi D**, Kim J, Yang JW, Kim JH, Park S, Shin JI. Dysregulated MicroRNAs in the Pathogenesis of Systemic Lupus Erythematosus: A Comprehensive Review. *Int J Biol Sci* 2023; **19**: 2495-2514 [PMID: 37215992 DOI: 10.7150/ijbs.74315]
13 **Zhang Y**, Chen X, Deng Y. miR-125a-3p decreases levels of interlukin-17 and suppresses renal fibrosis *via* down-regulating TGF-β1 in systemic lupus erythematosus mediated Lupus nephritic mice. *Am J Transl Res* 2019; **11**: 1843-1853 [PMID: 30972208]
14 **Chi M**, Ma K, Li Y, Quan M, Han Z, Ding Z, Liang X, Zhang Q, Song L, Liu C. Immunological Involvement of MicroRNAs in the Key Events of Systemic Lupus Erythematosus. *Front Immunol* 2021; **12**: 699684 [PMID: 34408748 DOI: 10.3389/fimmu.2021.699684]
15 **Zhao X**, Tang Y, Qu B, Cui H, Wang S, Wang L, Luo X, Huang X, Li J, Chen S, Shen N. MicroRNA-125a contributes to elevated inflammatory chemokine RANTES levels *via* targeting KLF13 in systemic lupus erythematosus. *Arthritis Rheum* 2010; **62**: 3425-3435 [PMID: 20589685 DOI: 10.1002/art.27632]
16 **Hu R**, O'Connell RM. MicroRNA control in the development of systemic autoimmunity. *Arthritis Res Ther* 2013; **15**: 202 [PMID: 23379780 DOI: 10.1186/ar4131]
17 **Cross JT**, Benton HP. The roles of interleukin-6 and interleukin-10 in B cell hyperactivity in systemic lupus erythematosus. *Inflamm Res* 1999; **48**: 255-261 [PMID: 10391113 DOI: 10.1007/s000110050456]
18 **Maczynska I**, Millo B, Ratajczak-Stefańska V, Maleszka R, Szych Z, Kurpisz M, Giedrys-Kalemba S. Proinflammatory cytokine (IL-1beta, IL-6, IL-12, IL-18 and TNF-alpha) levels in sera of patients with subacute cutaneous lupus erythematosus (SCLE). *Immunol Lett* 2006; **102**: 79-82 [PMID: 16154204 DOI: 10.1016/j.imlet.2005.08.001]
19 **Liu TF**, Jones BM, Wong RW, Srivastava G. Impaired production of IL-12 in systemic lupus erythematosus. III: deficient IL-12 p40 gene expression and cross-regulation of IL-12, IL-10 and IFN-gamma gene expression. *Cytokine* 1999; **11**: 805-811 [PMID: 10525320 DOI: 10.1006/cyto.1999.0512]
20 **Lauwerys BR**, Van Snick J, Houssiau FA. Serum IL-12 in systemic lupus erythematosus: absence of p70 heterodimers but presence of p40 monomers correlating with disease activity. *Lupus* 2002; **11**: 384-387 [PMID: 12139377 DOI: 10.1191/0961203302lu213oa]
21 **Larosa M**, Zen M, Gatto M, Jesus D, Zanatta E, Iaccarino L, Inês L, Doria A. IL-12 and IL-23/Th17 axis in systemic lupus erythematosus. *Exp Biol Med (Maywood)* 2019; **244**: 42-51 [PMID: 30664357 DOI: 10.1177/1535370218824547]
22 **Ye Q-L**, Guoliu R-N, Qin H, Shen Y-Y, Wang B, Zhai Z-MJIJCEP. Elevated plasma levels of IL-12 and IFN-γ in systemic lupus erythematosus. *Lupus* 2017; **10**: 3286-3291 [DOI: 10.1177/0961203316646462]
23 **La Cava A**. Anticytokine therapies in systemic lupus erythematosus. *Immunotherapy* 2010; **2**: 575-582 [PMID: 20636010 DOI: 10.2217/imt.10.29]

24 **Squatrito D**, Emmi G, Silvestri E, Ciucciarelli L, D'Elios MM, Prisco D, Emmi L. Pathogenesis and potential therapeutic targets in systemic lupus erythematosus: from bench to bedside. *Auto Immun Highlights* 2014; **5**: 33-45 [PMID: 26000154 DOI: 10.1007/s13317-014-0058-y]

25 **Svenungsson E**, Fei GZ, Jensen-Urstad K, de Faire U, Hamsten A, Frostegard J. TNF-alpha: a link between hypertriglyceridaemia and inflammation in SLE patients with cardiovascular disease. *Lupus* 2003; **12**: 454-461 [PMID: 12873047 DOI: 10.1191/0961203303lu412oa]

26 **Postal M**, Appenzeller S. The role of Tumor Necrosis Factor-alpha (TNF-α) in the pathogenesis of systemic lupus erythematosus. *Cytokine* 2011; **56**: 537-543 [PMID: 21907587 DOI: 10.1016/j.cyto.2011.08.026]

27 **Sabry A**, Sheashaa H, El-Husseini A, Mahmoud K, Eldahshan KF, George SK, Abdel-Khalek E, El-Shafey EM, Abo-Zenah H. Proinflammatory cytokines (TNF-alpha and IL-6) in Egyptian patients with SLE: its correlation with disease activity. *Cytokine* 2006; **35**: 148-153 [PMID: 17027280 DOI: 10.1016/j.cyto.2006.07.023]

28 **Dadashzadeh E**, Saghaeian Jazi M, Abdolahi N, Mohammadi S, Saeidi M. Comparison of a Suggested Model of Fibrosis in Human Dermal Fibroblasts by Serum from Systemic Sclerosis Patients with Transforming Growth Factor β Induced in vitro Model. *Int J Mol Cell Med* 2022; **11**: 31-40 [PMID: 36397805 DOI: 10.22088/IJMCM.BUMS.11.1.31]

29 **Aringer M**, Smolen JS. The role of tumor necrosis factor-alpha in systemic lupus erythematosus. *Arthritis Res Ther* 2008; **10**: 202 [PMID: 18226185 DOI: 10.1186/ar2341]

30 **Mohammadi S**, Sedighi S, Memarian A, Yazdani YJL. Overexpression of interferon-γ and indoleamine 2, 3-dioxygenase in systemic lupus erythematosus: relationship with the disease activity. *Laboratoriums Medizin* 2017; **41**: 41-47 [DOI: 10.1515/Labmed-2016-0076]

31 **Ahearn JM**, Liu CC, Kao AH, Manzi S. Biomarkers for systemic lupus erythematosus. *Transl Res* 2012; **159**: 326-342 [PMID: 22424435 DOI: 10.1016/j.trsl.2012.01.021]

32 **Banerjee S**, Cui H, Xie N, Tan Z, Yang S, Icyuz M, Thannickal VJ, Abraham E, Liu G. miR-125a-5p regulates differential activation of macrophages and inflammation. *J Biol Chem* 2013; **288**: 35428-35436 [PMID: 24151079 DOI: 10.1074/jbc.M112.426866]

33 **Ha TY**. The Role of MicroRNAs in Regulatory T Cells and in the Immune Response. *Immune Netw* 2011; **11**: 11-41 [PMID: 21494372 DOI: 10.4110/in.2011.11.1.11]

34 **Lee HM**, Kim TS, Jo EK. MiR-146 and miR-125 in the regulation of innate immunity and inflammation. *BMB Rep* 2016; **49**: 311-318 [PMID: 26996343 DOI: 10.5483/bmbrep.2016.49.6.056]

35 **Tili E**, Michaille JJ, Croce CM. MicroRNAs play a central role in molecular dysfunctions linking inflammation with cancer. *Immunol Rev* 2013; **253**: 167-184 [PMID: 23550646 DOI: 10.1111/imr.12050]

36 **Roohi A**, Yazdani Y, Khoshnoodi J, Jazayeri SM, Carman WF, Chamankhah M, Rashedan M, Shokri F. Differential reactivity of mouse monoclonal anti-HBs antibodies with recombinant mutant HBs antigens. *World J Gastroenterol* 2006; **12**: 5368-5374 [PMID: 16981270 DOI: 10.3748/wjg.v12.i33.5368]

37 **Yazdani Y**, Sadeghi H, Alimohammadian M, Andalib A, Moazen F, Rezaei A. Expression of an innate immune element (mouse hepcidin-1) in baculovirus expression system and the comparison of its function with synthetic human hepcidin-25. *Iran J Pharm Res* 2011; **10**: 559-568 [PMID: 24250389]

38 **Jazi MS**, Mohammadi S, Yazdani Y, Sedighi S, Memarian A, Aghaei M. Effects of valproic acid and pioglitazone on cell cycle progression and proliferation of T-cell acute lymphoblastic leukemia Jurkat cells. *Iran J Basic Med Sci* 2016; **19**: 779-786 [PMID: 27635203]

39 **Ghasemi H**, Jamshidi A, Ghatee MA, Mazhab-Jafari K, Khorasani M, Rahmati M, Mohammadi S. PPARγ activation by pioglitazone enhances the anti-proliferative effects of doxorubicin on pro-monocytic THP-1 leukemia cells *via* inducing apoptosis and G2/M cell cycle arrest. *J Recept Signal Transduct Res* 2022; **42**: 429-438 [PMID: 34645362 DOI: 10.1080/10799893.2021.1988972]

40 **Zhang C**, Wang X, Chen Y, Wu Z, Zhang C, Shi W. The down-regulation of hsa_circ_0012919, the sponge for miR-125a-3p, contributes to DNA methylation of CD11a and CD70 in CD4(+) T cells of systemic lupus erythematous. *Clin Sci (Lond)* 2018; **132**: 2285-2298 [PMID: 30237316 DOI: 10.1042/CS20180403]

41 **Nascimento DQ**, da Silva IIFG, Lima CAD, Cavalcanti AS, Roberti LR, Queiroz RGP, Ferriani VPL, Crovella S, Carvalho LM, Sandrin-Garcia P. Expression of the miR-9-5p, miR-125b-5p and its target gene NFKB1 and TRAF6 in childhood-onset systemic lupus erythematosus (cSLE). *Autoimmunity* 2022; **55**: 515-519 [PMID: 36177494 DOI: 10.1080/08916934.2022.2128781]

42 **Eissa E**, Morcos B, Abdelkawy RFM, Ahmed HH, Kholoussi NM. Association of microRNA-125a with the clinical features, disease activity and inflammatory cytokines of juvenile-onset lupus patients. *Lupus* 2021; **30**: 1180-1187 [PMID: 33866896 DOI: 10.1177/09612033211010328]

43 **Babania O**, Mohammadi S, Yaghoubi E, Sohrabi A, Sadat Seyedhosseini F, Abdolahi N, Yazdani Y. The expansion of CD14+ CD163+ subpopulation of monocytes and myeloid cells-associated cytokine imbalance; candidate diagnostic biomarkers for celiac disease (CD). *J Clin Lab Anal* 2021; **35**: e23984 [PMID: 34449925 DOI: 10.1002/jcla.23984]

44 **Tang S**. miR-125 is a Novel Biomarker in Pancreatic Cancer Patients. University of Pittsburgh, 2014 [DOI: 10.7150/jca.31361]

45 **Lv A**, Tu Z, Huang Y, Lu W, Xie B. Circulating exosomal miR-125a-5p as a novel biomarker for cervical cancer. *Oncol Lett* 2021; **21**: 54 [PMID: 33281965 DOI: 10.3892/ol.2020.12316]

46 **Zhang J**, Chen C, Fu H, Yu J, Sun Y, Huang H, Tang Y, Shen N, Duan Y. MicroRNA-125a-Loaded Polymeric Nanoparticles Alleviate Systemic Lupus Erythematosus by Restoring Effector/Regulatory T Cells Balance. *ACS Nano* 2020; **14**: 4414-4429 [PMID: 32203665 DOI: 10.1021/acsnano.9b09998]

47 **Idborg H**, Eketjäll S, Pettersson S, Gustafsson JT, Zickert A, Kvarnström M, Oke V, Jakobsson PJ, Gunnarsson I, Svenungsson E. TNF-α and plasma albumin as biomarkers of disease activity in systemic lupus erythematosus. *Lupus Sci Med* 2018; **5**: e000260 [PMID: 29955370 DOI: 10.1136/lupus-2018-000260]

48 **Ma CY**, Jiao YL, Zhang J, Yang QR, Zhang ZF, Shen YJ, Chen ZJ, Zhao YR. Elevated plasma level of HMGB1 is associated with disease activity and combined alterations with IFN-α and TNF-α in systemic lupus erythematosus. *Rheumatol Int* 2012; **32**: 395-402 [PMID: 21120500 DOI: 10.1007/s00296-010-1636-6]

49 **Rana A**, Minz RW, Aggarwal R, Anand S, Pasricha N, Singh S. Gene expression of cytokines (TNF-α, IFN-γ), serum profiles of IL-17 and IL-23 in paediatric systemic lupus erythematosus. *Lupus* 2012; **21**: 1105-1112 [PMID: 22759859 DOI: 10.1177/0961203312451200]

50 **Sabry A**, Elbasyouni SR, Sheashaa HA, Alhusseini AA, Mahmoud K, George SK, Kaleek EA, abo-Zena H, Kalil AM, Mohsen T, Rahim MA, El-samanody AZ. Correlation between levels of TNF-alpha and IL-6 and hematological involvement in SLE Egyptian patients with lupus nephritis. *Int Urol Nephrol* 2006; **38**: 731-737 [PMID: 17260180 DOI: 10.1007/s11255-006-0047-9]

51 **Aringer M**, Feierl E, Steiner G, Stummvoll GH, Höfler E, Steiner CW, Radda I, Smole JS, Graninger WB. Increased bioactive TNF in human systemic lupus erythematosus: associations with cell death. *Lupus* 2002; **11**: 102-108 [PMID: 11958572 DOI: 10.1191/0961203302lu160oa]

52 **Umare V**, Pradhan V, Nadkar M, Rajadhyaksha A, Patwardhan M, Ghosh KK, Nadkarni AH. Effect of proinflammatory cytokines (IL-6, TNF-α, and IL-1β) on clinical manifestations in Indian SLE patients. *Mediators Inflamm* 2014; **2014**: 385297 [PMID: 25548434 DOI: 10.1155/2014/385297]

53 **Zhu LJ**, Landolt-Marticorena C, Li T, Yang X, Yu XQ, Gladman DD, Urowitz MB, Fortin PR, Wither JE. Altered expression of TNF-alpha signaling pathway proteins in systemic lupus erythematosus. *J Rheumatol* 2010; **37**: 1658-1666 [PMID: 20516030 DOI: 10.3899/jrheum.091123]

54 **El-Zorkany BK**, Mahmoud GA, Shahin HA, Moustafa H, Shahin AA. Tumor necrosis factor-alpha and neuropsychiatric lupus erythematosus: relation to single photon emission computed tomography findings. *Mod Rheumatol* 2003; **13**: 250-255 [PMID: 24387213 DOI: 10.3109/s10165-003-0231-z]

55 **Postal M**, Lapa AT, Reis F, Rittner L, Appenzeller S. Magnetic resonance imaging in neuropsychiatric systemic lupus erythematosus: current state of the art and novel approaches. *Lupus* 2017; **26**: 517-521 [PMID: 28394232 DOI: 10.1177/0961203317691373]

56 **Capper ER**, Maskill JK, Gordon C, Blakemore AI. Interleukin (IL)-10, IL-1ra and IL-12 profiles in active and quiescent systemic lupus erythematosus: could longitudinal studies reveal patient subgroups of differing pathology? *Clin Exp Immunol* 2004; **138**: 348-356 [PMID: 15498048 DOI: 10.1111/j.1365-2249.2004.02607.x]

57 **Qiu F**, Song L, Yang N, Li X. Glucocorticoid downregulates expression of IL-12 family cytokines in systemic lupus erythematosus patients. *Lupus* 2013; **22**: 1011-1016 [PMID: 23884985 DOI: 10.1177/0961203313498799]

58 **Uzrail AH**, Assaf AM, Abdalla SS. Correlations of Expression Levels of a Panel of Genes (IRF5, STAT4, TNFSF4, MECP2, and TLR7) and Cytokine Levels (IL-2, IL-6, IL-10, IL-12, IFN-γ, and TNF-α) with Systemic Lupus Erythematosus Outcomes in Jordanian Patients. *Biomed Res Int* 2019; **2019**: 1703842 [PMID: 31871930 DOI: 10.1155/2019/1703842]

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ORIGINAL ARTICLE

Retrospective Cohort Study

Red cell distribution width: A predictor of the severity of hypertriglyceridemia-induced acute pancreatitis

Yong-Cai Lv, Yan-Hua Yao, Juan Zhang, Yu-Jie Wang, Jing-Jing Lei

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Yong-Cai Lv, Department of Gastroenterology, Zhenning Buyi and Miao Autonomous County People's Hospital, Zhenning 561200, Guizhou Province, China

Yan-Hua Yao, Juan Zhang, Yu-Jie Wang, Department of Gastroenterology, The Affiliated Baiyun Hospital of Guizhou Medical University, Guiyang 550014, Guizhou Province, China

Jing-Jing Lei, Department of Geriatric Medicine, The Affiliated Hospital of Guizhou Medical University, Guiyang 550014, Guizhou Province, China

Corresponding author: Jing-Jing Lei, PhD, Doctor, Department of Geriatric Medicine, The Affiliated Hospital of Guizhou Medical University, No. 28 Guiyi Street, Yunyan District, Guiyang 550004, Guizhou Province, China. lycyyh0325@163.com

Abstract

BACKGROUND

Compared with patients with other causes of acute pancreatitis, those with hypertriglyceridemia-induced acute pancreatitis (HTG-AP) are more likely to develop persistent organ failure (POF). Therefore, recognizing the individuals at risk of developing POF early in the HTG-AP process is a vital for improving outcomes. Bedside index for severity in acute pancreatitis (BISAP), a simple parameter that is obtained 24 h after admission, is an ideal index to predict HTG-AP severity; however, the suboptimal sensitivity limits its clinical application. Hence, current clinical scoring systems and biochemical parameters are not sufficient for predicting HTG-AP severity.

AIM

To elucidate the early predictive value of red cell distribution width (RDW) for POF in HTG-AP.

METHODS

In total, 102 patients with HTG-AP were retrospectively enrolled. Demographic and clinical data, including RDW, were collected from all patients on admission.

RESULTS

Based on the Revised Atlanta Classification, 37 (33%) of 102 patients with HTG-AP were diagnosed with POF. On admission, RDW was significantly higher in patients with HTG-AP and POF than in those without POF (14.4% *vs* 12.5%, $P < 0.001$). The receiver operating characteristic curve demonstrated a good discrim-

inative power of RDW for POF with a cutoff of 13.1%, where the area under the curve (AUC), sensitivity, and specificity were 0.85, 82.4%, and 77.9%, respectively. When the RDW was ≥ 13.1% and one point was added to the original BISAP to obtain a new BISAP score, we achieved a higher AUC, sensitivity, and specificity of 0.89, 91.2%, and 67.6%, respectively.

CONCLUSION
RDW is a promising predictor of POF in patients with HTG-AP, and the addition of RDW can promote the sensitivity of BISAP.

Key Words: Red cell distribution width; Bedside index for severity in acute pancreatitis; Persistent organ failure; Hypertriglyceridemia-induced acute pancreatitis

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Core Tip: Red cell distribution width (RDW) reflects systemic inflammation, which is significantly associated with the severity of acute pancreatitis. However, the relationship between RDW and hypertriglyceridemia-induced acute pancreatitis (HTG-AP) remains unclear. Herein, RDW exhibited a potent discriminatory power for predicting persistent organ failure in patients with HTG-AP. Furthermore, the addition of RDW is able to promote the sensitivity of bedside index for severity in acute pancreatitis.

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INTRODUCTION

Hypertriglyceridemia is the most common etiology of acute pancreatitis (AP)[1-3]. Compared with patients with other causes of AP, those with hypertriglyceridemia-induced AP (HTG-AP) are more likely to suffer from persistent organ failure (POF)[4,5]. POF is a major determinant of mortality in early HTG-AP and correlates with the magnitude of inflammatory responses[1], thereby indicating the prognosis of patients. Given this, early recognition of HTG-AP patients at risk of POF is necessary for improving patients' outcomes.

Red cell distribution width (RDW) reflects the size of circulating erythrocytes[6]. Studies[7,8] have reported that high RDW is significantly associated with AP severity. The mechanism underlying this association may be attributed to the following: (1) Inflammation blocks anti-apoptosis or promotes erythrocyte death[9]; (2) inflammatory cytokines can desensitize bone marrow erythroid progenitors to erythropoiesis, thereby inhibiting erythrocyte maturation[10]; and (3) during AP, erythrocytes cannot absorb vital materials, including vitamin B12, iron, and folic acid[11].

However, the relationship between RDW and HTG-AP was not well elucidated in previous studies; therefore, our study aimed to evaluate the predictive value of RDW for POF in patients with HTG-AP.

MATERIALS AND METHODS

Patient selection

In this retrospective study, consecutive patients with HTG-AP who were hospitalized at the Affiliated Baiyun Hospital of Guizhou Medical University from January 2017 to February 2021 were enrolled. AP and POF (lasting > 48 h) were diagnosed based on the Revised Atlanta Classification[12]. This study was reviewed and approved by the Science and Research Office of the Affiliated Baiyun Hospital of Guizhou Medical University. Patients' consent for inclusion was waived owing to the retrospective nature of the study.

HTG-AP was diagnosed if serum triglyceride levels were > 11.3 mmol/L or between 5.56 and 11.30 mmol/L accompanied by chylous effusion, with the exclusion of other etiologies[13,14].

The exclusion criteria were as follows: (1) Patients who were admitted > 24 h after AP onset, (2) those who were < 18 years of age; and (3) those with other etiology-induced AP, recurrent AP, gestational AP, AP with anemia (woman < 110 g/L, man < 120 g/L), chronic pancreatitis, and cancer history. The primary endpoint was in-hospital POF.

Data collection

All data were collected from our hospital's electronic medical database. For each patient, age, sex, and medical history were collected as baseline demographic data. Furthermore, information on vital signs was collected from all patients on admission, and the results of laboratory tests, radiological imaging, and clinical outcomes were collected within 24 h of

admission. The bedside index for severity in acute pancreatitis (BISAP)[15] and the Acute Physiology and Chronic Health Evaluation II (APACHE II) score[16] were calculated within 24 h of admission.

The measurement of RDW

On admission, 2-3 mL of blood was collected, placed in EDTA-K2 anticoagulation tubes, and stored at 4-8℃. The whole blood samples were directly loaded into a hematology analyzer (XN-2000, Japan Sysmex) for detection. RDW (%) = SD of red blood cell volume/mean corpuscular volume x 100. The reference range of RDW was 11.0%-16.0%.

Statistical analysis

Data were presented as median (interquartile range) or mean ± SD. Categorical data were presented as percentages (%). Univariate analysis was performed using the Student's *t*-test, Mann-Whitney U test, and chi-squared test, as appropriate. Admission variables that were significant in univariate analysis were subjected to multivariate regression analysis.

A receiver-operating characteristic (ROC) curve was used to estimate predictive accuracy. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) were calculated. Pearson's analysis was performed to determine the correlations between two variables. GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, United States) and SPSS 25.0 (IBM Corp., Armonk, NY, United States) were used to perform statistical analyses. A P value < 0.05 was considered statistically significant.

RESULTS

Patient demographics and clinical characteristics

Table 1 summarizes the demographic, laboratory, and clinical characteristics of patients with and without POF. In total, 102 patients were included in this retrospective study. The age range was 20-58 years and 34 (33%) of 102 patients developed POF; no patients died.

Clinical outcomes

Univariate analysis identified several risk factors of POF on admission (P < 0.05). Multivariate analysis revealed that RDW (P = 0.002) and BISAP (P = 0.001) were associated with POF (Table 1). RDW was positively correlated with BISAP (r = 0.457, P < 0.001) and APACHE II (r = 0.440, P < 0.001) (Figure 1).

ROC analysis indicated that the optimal cutoff value for RDW was 13.1%. The sensitivity, specificity, PPV, and NPV were 82.4%, 77.9%, 65.1%, and 89.8%, respectively. Using the cutoff for RDW, the 102 patients with HTG-AP were divided into two groups: RDW < 1 3.1% and RDW ≥ 13.1% groups. More POF patients were presents in the RDW ≥ 13.1% group (P < 0.001) (Figure 2 and Table 2).

When RDW was ≥ 13.1%, one point was added to the BISAP score to obtain a new BISAP score (BISAP plus RDW: BR score). When the cutoff value of BR score was set as 2 points, the AUC, sensitivity, specificity, PPV, and NPV for predicting POF was 0.89, 91.2%, 67.6%, 58.5%, and 93.9%, respectively (Figure 3 and Table 2). Compared with BISAP alone (AUC = 0.82, sensitivity = 64.7%, specificity = 82.4%, PPV = 64.7%, and NPV = 82.4%) and APACHE II (AUC = 0.74, sensitivity = 73.5%, specificity = 60.3%, PPV = 48.1%, and NPV = 82.0%), BR score exhibited a higher predictive accuracy (Figure 3 and Table 2).

DISCUSSION

Herein, our study suggested that RDW on admission was a promising indicator for POF and that the BR score could efficiently predict POF in patients with HTG-AP. This is the first study to uncover the association between RDW and POF in patients with HTG-AP.

Compared with other AP etiologies, HTG-AP is more likely to causes POF, which results in an unfavorable prognosis for patients[12,17,18]. Therefore, the predictive value of RDW is of significance because the application of this biomarker can facilitate the discrimination of patients who have a high risk of developing POF and the design of treatment regimens. Moreover, RDW examination is reproducible, inexpensive, and convenient, and thus can be widely implemented in almost every hospital.

The production of inflammatory cytokines and corresponding inflammatory cascade have long been considered a critical pathogenic mechanism of POF in AP patients[1,8]. RDW reflects systemic inflammation, and several studies[6-8] have proposed the RDW can predict in-hospital mortality and severity in AP. However, these studies only included gallstones- and alcohol-induced AP but not HTG-AP. Consequently, our study focused on the association between RDW and POF in patients with HTG-AP. The results indicated that RDW was positively correlated with BISAP and APACHE II scores, which were commonly used indicators of POF. Meanwhile, RDW exhibited a promising value in predicting POF.

Although the mechanism underlying increased RDW in HTG-AP patients developing POF, it may be attributed to factors as follows: inflammatory cascade triggers triglyceride-mediated lipotoxicity; in turn, increased triglyceride levels augment the intensity of the inflammatory cascade, which contributes to the elevation of RDW[17,18]. Notably, we excluded patients with anemia, which would affect RDW; this might promote the predictive value of RDW[6,9,19].

Table 1 Demographic and clinical characteristics of the patients, *N* (%)

Variable	Non-POF (*n* = 68)	POF (*n* = 34)	Univariate *P*	Multivariate *P*
Age (yr)	40 ± 8	40 ± 9	0.967	
Sex (M/F)	56/12	29/5	0.785	
Hypertension	16 (24)	7 (21)	0.876	
Diabetes	33 (49)	22 (65)	0.144	
Fatty liver disease	39 (57)	16 (47)	0.679	
Alcohol	28 (41)	15 (44)	0.833	
Smoking	45 (66)	21 (62)	0.667	
WBC (x10^9/L)	12.4 (10.9, 15.9)	14.9 (13.2, 17.1)	0.009	0.172
MPV (fL)	10.42 ± 1.76	11.54 ± 2.06	0.012	0.085
RDW (%)	12.5 (12.0, 13.0)	14.4 (13.4, 14.9)	< 0.001	0.002
Amylase (U/L)	124 (66, 258)	239 (83, 917)	0.036	0.383
Calcium (mmol/L)	2.35 (2.25, 2.42)	2.29 (2.10, 2.95)	0.064	
Triglyceride (mmol/L)	16.42 (11.58, 20.31)	19.57 (15.23, 24.70)	0.010	0.101
ALT (U/L)	36.0 (25.0, 47.4)	35.2 (20.8, 46.6)	0.412	
AST (U/L)	25.9 (19.1, 31.7)	25.4 (17.9, 33.6)	0.879	
Albumin (g/L)	46.1 (44.1, 48.4)	44.8 (42.1, 48.3)	0.078	
BUN (mmol/L)	4.30 (3.60, 5.18)	3.85 (2.90, 5.00)	0.057	
Creatinine (μmol/l)	60.50 (47.50, 76.55)	54.90 (41.70, 71.05)	0.158	
C-reaction protein (mg/L)	24.89 (11.14, 57.54)	63.10 (23.57, 99.91)	0.008	0.185
BISAP	1 (0, 1)	2 (1, 2)	< 0.001	0.001
APACHE II	4 (2, 5)	5 (4, 8)	< 0.001	0.119

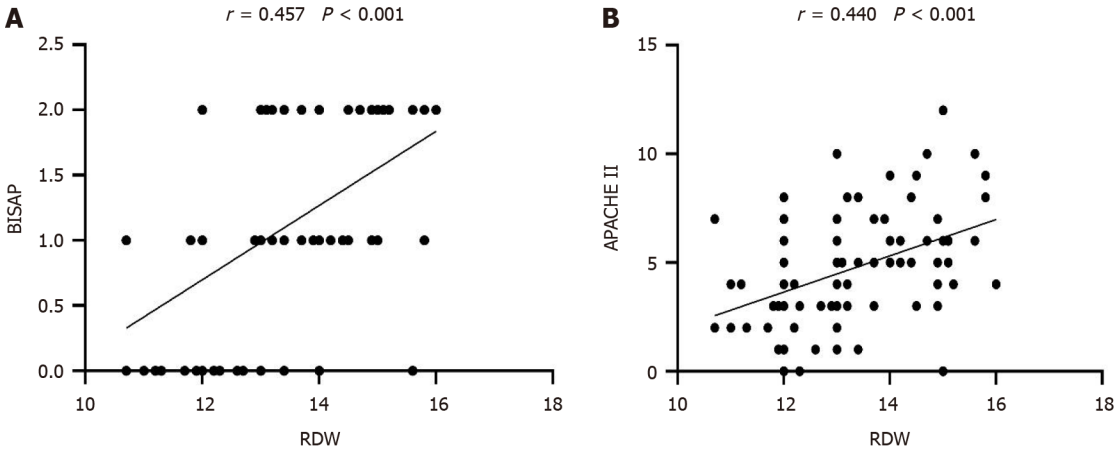
Data are presented as *n* (%) prevalence, and mean ± SD or median (interquartile range). WBC: White blood cells; MPV: Mean platelet volume; RDW: Red cell distribution width; ALT: Alanine aminotransferase; AST: Aspartate transaminase; BUN: Blood urea nitrogen; BISAP: Bedside index for severity in acute pancreatitis; APACHE II: Acute Physiology and Chronic Health Evaluation II; POF: Persistent organ failure.

Table 2 Overall accuracy of red cell distribution width (RDW), bedside index for severity in acute pancreatitis (BISAP), acute physiology and chronic health evaluation II, and BISAP plus RDW score for predicting persistent organ failure in patients with hypertriglyceridemia-induced acute pancreatitis on admission

Variable	AUC	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
RDW	0.85	13.1	82.4	77.9	65.1	89.8
BISAP	0.82	2	64.7	82.4	64.7	82.4
APACHE II	0.74	5	73.5	60.3	48.1	82.0
BR	0.89	2	91.2	67.6	58.5	93.9

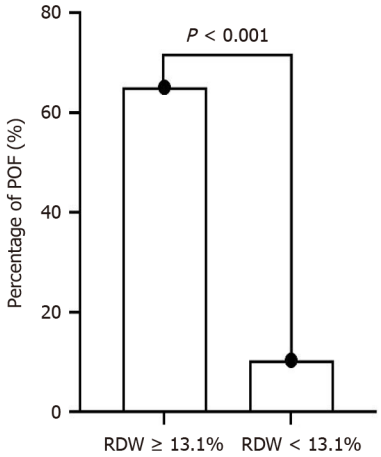
HTG-AP: Hypertriglyceridemia-induced acute pancreatitis; AUC: Area under the curve; PPV: Positive predictive value; PPV: Negative predictive value; RDW: Red cell distribution width; BISAP: Bedside index for severity in acute pancreatitis; APACHE II: Acute Physiology and Chronic Health Evaluation II; BR: BISAP plus RDW.

Several multiparameter predictors, including Ranson criteria, BISAP, and APACHE II, have been adopted to evaluate POF in HTG-AP patients[20-22]. However, Ranson criteria can only be used after 48 h of hospitalization and the calculation of APACHE II is complicated[16]. Although BISAP is widely used because of its simplicity of calculation, its low sensitivity limits its clinical application[16]. Li *et al*[21] compared the differences in scoring systems for predicting the prognosis of HTG-AP patients. They suggested that BISAP was the best indicator for predicting the HTG-AP severity. However, BISAP had a suboptimal sensitivity (66.7%) for predicting POF. Similarly, Yang *et al*[20] reported that BISAP exhibited low sensitivity (54%) for predicting HTG-AP severity. Consistent with previous findings, our results revealed



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Figure 1 Correlation between red cell distribution width and bedside index for severity in acute pancreatitis and acute physiology and chronic health evaluation II. A: Positive correlation between red cell distribution width (RDW) and bedside index for severity in acute pancreatitis; B: Positive correlation between RDW and acute physiology and chronic health evaluation II. RDW: Red cell distribution width; BISAP: Bedside index for severity in acute pancreatitis; APACHE-II: Acute Physiology and Chronic Health Evaluation II.



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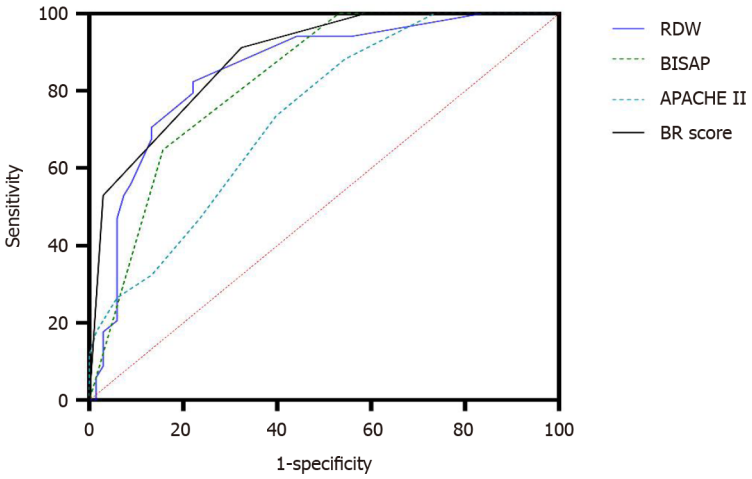
Figure 2 Percentage of patients with hypertriglyceridemia-induced acute pancreatitis between the red cell distribution width (RDW) < 13.1% group and the RDW ≥ 13.1% group. POF: Persistent organ failure; RDW: Red cell distribution width.

that BISAP also exhibited a low sensitivity (64.7%) for predicting POF.

Several studies have demonstrated the improved predictive value by combining BISAP and other biomarkers. Zheng *et al*[22] found that BISAP plus C-reactive protein levels for predicting POF in AP had increased AUC, sensitivity, and specificity compared with using BISAP alone (0.873, 81.6%, and 85.2% *vs* 0.856, 80.8%, and 84.1%, respectively). Zhou *et al* [19] (BISAP plus RDW) also suggested that while the addition of RDW promoted the predictive value of BISAP, it decreased the sensitivity. In the present study, the results indicated that the BR score exhibited higher AUC (0.89 *vs* 0.82) and sensitivity (91.2% *vs* 64.7%) than BISAP alone for predicting POF in HTG-AP patients, whereas the specificity (67.6% *vs* 82.4%) dropped.

Fluid therapy is a long-established cornerstone to prevent organ hypoperfusion within 24 h of AP onset[16,23]. Recent studies[17,23] have indicated that aggressive fluid therapy in AP may result in poor outcomes, particularly in older patients. Since HTG-AP patients tend to have a younger age, aggressive fluid therapy is a relatively safe treatment. However, our results suggested that more attention should be paid to patients with RDW ≥ 13.1%.

Our study has some limitations. First, no patient died in our cohort; therefore, the ability of RDW to predict mortality could not be investigated. However, considering the intimate association between POF development and unfavorable prognosis at the early stage of AP[12], we inferred that the mortality prediction performance of RDW might be superior in HTG-AP than in POF. Second, this study was limited by its retrospective design and small sample size, making it impossible to draw definitive conclusions regarding the diagnostic value of evaluated biomarkers for predicting POF in HTG-AP. Third, the definition of HTG-AP differed between countries[17], which limited the extension of our findings to other countries. Moreover, we did not perform a subgroup analysis to exclude the influence of metabolic disorders on RDW. Furthermore, BR score had a high false positive rate, indicating that the BR score was not an optimal indicator for



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Figure 3 Receiver operating characteristic curves for red cell distribution width (RDW), bedside index for severity in acute pancreatitis (BISAP), Acute Physiology and Chronic Health Evaluation II, and BISAP plus RDW score for predicting persistent organ failure in hypertriglyceridemia-induced acute pancreatitis. HTG-AP: Hypertriglyceridemia-induced acute pancreatitis; RDW: Red cell distribution width; BISAP: Bedside index for severity in acute pancreatitis; APACHE II: Acute Physiology and Chronic Health Evaluation II; BR: BISAP plus RDW; POF: Persistent organ failure.

predicting POF. Nevertheless, the BR score had a relatively high sensitivity, so it could be a screening indicator for POF in HTG-AP patients. Additional new biomarkers and scoring systems should be developed to better predict POF.

CONCLUSION

RDW on admission is an independent predictor of POF in patients with HTG-AP. The addition of RDW improves the low sensitivity of BISAP. Large-scale, multicenter prospective studies are required to verify the results.

ARTICLE HIGHLIGHTS

Research background

In clinical settings, compared with patients with other causes of acute pancreatitis (AP), those with hypertriglyceridemia-induced acute pancreatitis (HTG-AP) commonly suffer from severe acute pancreatitis; therefore, it is critical to identify severe HTG-AP early. However, current clinical scoring systems and biochemical parameters are not efficient for predicting HTG-AP severity.

Research motivation

Red cell distribution width (RDW) may be closely associated with the mortality of patients with AP. Meanwhile, clinical application of the bedside index for severity in acute pancreatitis (BISAP) is limited by its low sensitivity. Therefore, new parameters or scoring systems are warranted for determining HTG-AP severity early.

Research objectives

To determine whether RDW can be used as a potential biomarker for predicting POF in HTG-AP.

Research methods

We explored the relationship between RDW and POF in patients with HTG-AP and determined the cutoff value of RDW using ROC analysis. BISAP plus RDW improved the suboptimal sensitivity of BISAP when RDW was ≥ 13.1% and one point was added to the BISAP.

Research results

On admission, RDW was significantly higher in patients with HTG-AP and POF. Compared with BISAP and Ranson criteria, BISAP plus RDW had a higher accuracy for predicting POF in HTG-AP patients.

Research conclusions

BISAP plus RDW exhibited a promising predictive value for POF in HTG-AP patients, despite its low specificity.

Research perspectives

The combination of BISAP and other indicators may better predict HTG-AP severity. However, additional large-scale, multicenter prospective studies are required to verify the results.

FOOTNOTES

Author contributions: Lv YC and Lei JJ designed the study; Lv YC, Yao YH, Zhang J, and Wang YJ, participated in the acquisition, analysis, and interpretation of the data; Lv YC wrote the manuscript; Lei JJ revised the article.

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Institutional review board statement: This study was reviewed and approved by the Science and Research Office of the Affiliated Baiyun Hospital of Guizhou Medical University.

Informed consent statement: Patients' consent for inclusion was waived owing to the retrospective nature of the study.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: No additional data are available.

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: China

ORCID number: Yong-Cai Lv 0000-0001-8893-4315; Yan-Hua Yao 0000-0002-3515-4929; Juan Zhang 0000-0001-9280-9082; Yu-Jie Wang 0000-0002-7845-805X; Jing-Jing Lei 0000-0003-2293-5272.

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REFERENCES

1 **Tsuang W**, Navaneethan U, Ruiz L, Palascak JB, Gelrud A. Hypertriglyceridemic pancreatitis: presentation and management. *Am J Gastroenterol* 2009; **104**: 984-991 [PMID: 19293788 DOI: 10.1038/ajg.2009.27]
2 **Yadav D**, Lowenfels AB. Trends in the epidemiology of the first attack of acute pancreatitis: a systematic review. *Pancreas* 2006; **33**: 323-330 [PMID: 17079934 DOI: 10.1097/01.mpa.0000236733.31617.52]
3 **Guo YY**, Li HX, Zhang Y, He WH. Hypertriglyceridemia-induced acute pancreatitis: progress on disease mechanisms and treatment modalities. *Discov Med* 2019; **27**: 101-109 [PMID: 30939294]
4 **Huang YX**, Jia L, Jiang SM, Wang SB, Li MX, Yang BH. Incidence and clinical features of hyperlipidemic acute pancreatitis from Guangdong, China: a retrospective multicenter study. *Pancreas* 2014; **43**: 548-552 [PMID: 24717803 DOI: 10.1097/MPA.0000000000000069]
5 **Deng LH**, Xue P, Xia Q, Yang XN, Wan MH. Effect of admission hypertriglyceridemia on the episodes of severe acute pancreatitis. *World J Gastroenterol* 2008; **14**: 4558-4561 [PMID: 18680239 DOI: 10.3748/wjg.14.4558]
6 **Cetinkaya E**, Senol K, Saylam B, Tez M. Red cell distribution width to platelet ratio: new and promising prognostic marker in acute pancreatitis. *World J Gastroenterol* 2014; **20**: 14450-14454 [PMID: 25339831 DOI: 10.3748/wjg.v20.i39.14450]
7 **Goyal H**, Awad H, Hu ZD. Prognostic value of admission red blood cell distribution width in acute pancreatitis: a systematic review. *Ann Transl Med* 2017; **5**: 342 [PMID: 28936436 DOI: 10.21037/atm.2017.06.61]
8 **Şenol K**, Saylam B, Kocaay F, Tez M. Red cell distribution width as a predictor of mortality in acute pancreatitis. *Am J Emerg Med* 2013; **31**: 687-689 [PMID: 23399348]
9 **Krichenko A**, Bueno RR, Andrade PM, Cunha CP, Souza AM, Ribeiro EJ, Pereira MA, Rossi PR, Carvalho RG, Brofman PR. Aortic coarctation in an infant treated with balloon catheter. A case report. *Arq Bras Cardiol* 1986; **46**: 349-351 [PMID: 2952106 DOI: 10.17219/acem/67590]
10 **Förhécz Z**, Gombos T, Borgulya G, Pozsonyi Z, Prohászka Z, Jánoskuti L. Red cell distribution width in heart failure: prediction of clinical events and relationship with markers of ineffective erythropoiesis, inflammation, renal function, and nutritional state. *Am Heart J* 2009; **158**: 659-666 [PMID: 19781428 DOI: 10.1016/j.ahj.2009.07.024]
11 **Lippi G**, Targher G, Montagnana M, Salvagno GL, Zoppini G, Guidi GC. Relation between red blood cell distribution width and inflammatory

biomarkers in a large cohort of unselected outpatients. *Arch Pathol Lab Med* 2009; **133**: 628-632 [PMID: 19391664 DOI: 10.5858/133.4.628]

12 **Banks PA**, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, Tsiotos GG, Vege SS; Acute Pancreatitis Classification Working Group. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 2013; **62**: 102-111 [PMID: 23100216 DOI: 10.1136/gutjnl-2012-302779]

13 **Scherer J**, Singh VP, Pitchumoni CS, Yadav D. Issues in hypertriglyceridemic pancreatitis: an update. *J Clin Gastroenterol* 2014; **48**: 195-203 [PMID: 24172179 DOI: 10.1097/01.mcg.0000436438.60145.5a]

14 **de Pretis N**, Amodio A, Frulloni L. Hypertriglyceridemic pancreatitis: Epidemiology, pathophysiology and clinical management. *United European Gastroenterol J* 2018; **6**: 649-655 [PMID: 30083325 DOI: 10.1177/2050640618755002]

15 **Wu BU**, Johannes RS, Sun X, Tabak Y, Conwell DL, Banks PA. The early prediction of mortality in acute pancreatitis: a large population-based study. *Gut* 2008; **57**: 1698-1703 [PMID: 18519429 DOI: 10.1136/gut.2008.152702]

16 **Mederos MA**, Reber HA, Girgis MD. Acute Pancreatitis: A Review. *JAMA* 2021; **325**: 382-390 [PMID: 33496779 DOI: 10.1001/jama.2020.20317]

17 **Carr RA**, Rejowski BJ, Cote GA, Pitt HA, Zyromski NJ. Systematic review of hypertriglyceridemia-induced acute pancreatitis: A more virulent etiology? *Pancreatology* 2016; **16**: 469-476 [PMID: 27012480 DOI: 10.1016/j.pan.2016.02.011]

18 **Nawaz H**, Koutroumpakis E, Easler J, Slivka A, Whitcomb DC, Singh VP, Yadav D, Papachristou GI. Elevated serum triglycerides are independently associated with persistent organ failure in acute pancreatitis. *Am J Gastroenterol* 2015; **110**: 1497-1503 [PMID: 26323188 DOI: 10.1038/ajg.2015.261]

19 **Zhou H**, Mei X, He X, Lan T, Guo S. Severity stratification and prognostic prediction of patients with acute pancreatitis at early phase: A retrospective study. *Medicine (Baltimore)* 2019; **98**: e15275 [PMID: 31008971 DOI: 10.1097/MD.0000000000015275]

20 **Yang L**, Liu J, Xing Y, Du L, Chen J, Liu X, Hao J. Comparison of BISAP, Ranson, MCTSI, and APACHE II in Predicting Severity and Prognoses of Hyperlipidemic Acute Pancreatitis in Chinese Patients. *Gastroenterol Res Pract* 2016; **2016**: 1834256 [PMID: 27882045 DOI: 10.1155/2016/1834256]

21 **Li M**, Xing XK, Lu ZH, Guo F, Su W, Lin YJ, Wang DH. Comparison of Scoring Systems in Predicting Severity and Prognosis of Hypertriglyceridemia-Induced Acute Pancreatitis. *Dig Dis Sci* 2020; **65**: 1206-1211 [PMID: 31515723 DOI: 10.1007/s10620-019-05827-9]

22 **Zheng J**, Zhang J, Gao J. Early evaluations of BISAP plus C-reactive protein in predicting the severity of acute pancreatitis. *Zhonghua Yi Xue Za Zhi* 2015; **95**: 925-928 [PMID: 26081056 DOI: 10.3760/cma.j.issn.0376-2491.2015.12.012]

23 **de-Madaria E**, Soler-Sala G, Sánchez-Payá J, Lopez-Font I, Martínez J, Gómez-Escolar L, Sempere L, Sánchez-Fortún C, Pérez-Mateo M. Influence of fluid therapy on the prognosis of acute pancreatitis: a prospective cohort study. *Am J Gastroenterol* 2011; **106**: 1843-1850 [PMID: 21876561 DOI: 10.1038/ajg.2011.236]

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ORIGINAL ARTICLE

Observational Study

Ground level utility of Access, Watch, Reserve classification: Insights from a tertiary care center in North India

Gunjita Negi, Arjun KB, Prasan Kumar Panda

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Gunjita Negi, Arjun KB, Prasan Kumar Panda, Department of Medicine, All India Institute of Medical Sciences, Rishikesh, Rishikesh 249203, India

Corresponding author: Prasan Kumar Panda, MBBS, MD, Associate Professor, Department of Medicine, All India Institute of Medical Sciences, Room No. 409, Rishikesh 249203, India. motherprasanna@rediffmail.com

Abstract

BACKGROUND

The overuse and misuse of antimicrobials contribute significantly to antimicrobial resistance (AMR), which is a global public health concern. India has particularly high rates of AMR, posing a threat to effective treatment. The World Health Organization (WHO) Access, Watch, Reserve (AWaRe) classification system was introduced to address this issue and guide appropriate antibiotic prescribing. However, there is a lack of studies examining the prescribing patterns of antimicrobials using the AWaRe classification, especially in North India. Therefore, this study aimed to assess the prescribing patterns of antimicrobials using the WHO AWaRe classification in a tertiary care centre in North India.

AIM

To study the prescribing patterns of antimicrobials using WHO AWaRe classification through a cross-sectional study in All India Institute of Medical Sciences Rishikesh.

METHODS

A descriptive, cross-sectional study was conducted from July 2022 to August 2022 at a tertiary care hospital. Prescriptions containing at least one antimicrobial were included in the study. Data on prescriptions, including patient demographics, departments, types of antimicrobials prescribed, and duration of treatment, were collected. A questionnaire-based survey was also conducted to assess the knowledge and practices of prescribing doctors regarding the utility of AWaRe classification.

RESULTS

The study involved a total of 123 patients, each of whom received at least one antimicrobial prescription. Most prescriptions were for inpatients, evenly distributed between Medicine (Internal medicine, Pediatrics, Dermatology) and Surgical departments (General surgery and specialties, Otorhinolaryngology,

Ophthalmology, Obstetrics and Gynecology). Metronidazole and ceftriaxone were the most prescribed antibiotics. According to the AWaRe classification, 57.61% of antibiotics fell under the Access category, 38.27% in Watch, and 4.11% in Reserve. Most Access antibiotics were prescribed within the Medicine department, and the same department also exhibited a higher frequency of Watch antibiotics prescriptions. The questionnaire survey showed that only a third of participants were aware of the AWaRe classification, and there was a lack of knowledge regarding AMR and the potential impact of AWaRe usage.

CONCLUSION

This study highlights the need for better antimicrobial prescribing practices and increased awareness of the WHO AWaRe classification and AMR among healthcare professionals. The findings indicate a high proportion of prescriptions falling under the Access category, suggesting appropriate antibiotic selection. However, there is a significant difference between the WHO Defined Daily Dose and the prescribed daily dose in the analysed prescriptions suggesting overuse and underuse of antibiotics. There is room for improvement and educational interventions and antimicrobial stewardship programs should be implemented to enhance knowledge and adherence to guidelines, ultimately contributing to the containment of AMR.

Key Words: Antimicrobial resistance; AWaRe classification; Access; Watch; Reserve; Daily defined dose; Questionnaire based survey

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Core Tip: With the rise in antimicrobial resistance (AMR) particularly in developing countries it is the need of the hour to adopt better prescribing practices. It is important to raise knowledge and awareness about AMR and improve adherence to guidelines. The research highlights areas of improvement in prescribing practices.

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INTRODUCTION

The discovery of antimicrobials is regarded as one of the most crucial advancements of the 20th century. It is imperative to prescribe antimicrobials judiciously, emphasizing their use in circumstances where they can offer significant advantages to patients with maximum efficiency, all the while mitigating the potential for exacerbating antimicrobial resistance (AMR)[1]. However, there is mounting evidence indicating that the overuse and misuse of antimicrobials have become leading factors in the complex causative web of AMR[2]. It poses a global public health problem, capable of spreading and causing significant human and economic burdens[3]. The situation in India is particularly concerning, as the country experiences some of the highest rates of AMR among bacteria commonly causing infections in both community and healthcare settings[4].

It is imperative to address the emergence of Multi-drug resistant organisms (MDR), which threaten the progress made in medical advancements over the past century. Conversely, in many parts of the world, there is not only overuse and misuse of antimicrobials but also inadequate access. Pneumonia remains a leading cause of childhood deaths globally, with over 2 million estimated deaths per year, primarily due to limited access to antimicrobials[5]. Thus, promoting access to these life-saving medicines for those in need should also be a priority[1].

Antimicrobial stewardship aims to bridge the gap between excessive and inadequate antimicrobial use[6]. However, assessing the impact of antimicrobial stewardship programs has proven challenging, as the commonly used metrics, such as defined daily doses (DDD) per 1000 inhabitants per day and days of therapy, provide limited information about the quality of antibiotic use[7]. The existing defined daily dose method used in adult antibiotic surveillance is unsuitable for neonates and children due to their widely variable bodyweights[8].

To address these issues, the World Health Organization (WHO) updated its Model List of Essential Medicines in 2017, introducing the Access, Watch, Reserve (AWaRe) classification system[9]. The AWaRe system categorizes antimicrobials based on their appropriateness, safety, and potential impact on AMR. The Access group includes antibiotics of choice for the 25 most common infections, with favourable safety profiles and a low likelihood of exacerbating AMR. These antimicrobials should be consistently available, affordable, and quality-assured. The Watch group comprises critically important antimicrobials that require strict monitoring and limited use due to their higher potential for negatively impacting AMR. The Reserve group includes last-resort antimicrobials effective against MDR bacteria, and their use should be minimized as they represent a valuable, non-renewable resource. The fourth category is of the discouraged antimicrobials which mostly includes antimicrobial combinations. Some fixed dose combinations of antibiotics, do not have any reasonable

indications for the treatment of infectious diseases in humans and may negatively impact AMR and patient safety[1]. The AWaRe system is also represented as a traffic-light approach: Access = green, Watch = orange and Reserve = red. The overall goal was to reduce the use of Watch Group and Reserve Group, and to increase the use of Access antibiotics to > 60% by 2023[5].

Unlike previous measures of antibiotic consumption, the AWaRe classification allows for the quantification of antibiotic use in each category, providing insights into the overall quality of antibiotic use within a country[8]. For instance, A 10-Year Study on urinary tract infections, their Epidemiology and Antibiotic Resistance Based on the WHO AWaRe classification was done[10]. Therefore, the WHO AWaRe categories can serve as a valuable tool for monitoring antibiotic consumption and optimizing antibiotic use, complementing antibiotic stewardship efforts at the national level.

Given the ongoing struggle to identify appropriate measures for hospitals and their antimicrobial stewardship programs, this study aims to audit antimicrobial prescriptions and compare the utility of the AWaRe classification with other process measurements of antimicrobial utilization. Additionally, the study seeks to assess the knowledge and attitudes of prescribing physicians regarding AMR and the AWaRe classification. This study is particularly important in the context of North India, where the burden of resistance is increasing, and limited research has been conducted in this region. Furthermore, the study aims to examine the prescribing practices of antibiotics in a tertiary care center and compare them with the WHO Defined Daily Dosage to determine whether common antibiotics are being under or over-dosed.

MATERIALS AND METHODS

Study design

Cross sectional study conducted on patient's prescriptions of various departments in a tertiary care hospital from July 2022 to August 2022.

Setting

This study was carried out in a tertiary care centre in North India after approval by the Institutional Ethics Committee [All India Institute of Medical Sciences, Rishikesh, India (Reference number -AIIMS/IEC/22/252)].

Study population

Consenting treating physicians (faculty, junior and senior residents) and their prescriptions having at least one antimicrobial were studied. Universal sampling was used to select prescriptions for a duration of 2 mo.

Inclusion criteria

Prescriptions containing at least one antimicrobial in both outpatient and inpatient settings and the physician prescribing it.

Exclusion criteria

Treating physician not giving consent.

Observations

After obtaining an informed consent from the prescribing doctor, a questionnaire about their knowledge and practices on AWaRe classification was asked through online or offline modes. The questionnaire was self-structured after searching medical literature for comparable studies and adapting questions designed in other physicians' surveys previously carried out[1,11]. The questionnaire consisted of two sets of questions: one designed to assess the knowledge of physicians and the other intended to gauge their attitudes towards AMR and the AWaRe classification. Pre-validation of questionnaire for its contents and relevance was done by experts (one clinician, one pharmacologist, one biostatistician). Data on prescriptions, including patient demographics, departments, types of antimicrobials prescribed, and duration of treatment, were collected.

Comparator

The relevance of antimicrobial prescriptions was checked by measuring appropriateness (right drug, right dose and right duration) according to WHO guidelines, Infectious Disease Society of America guidelines and disease specific guidelines if needed. Simultaneously sub-group comparison on AWaRE classification was also performed.

Outcome

Proportion of prescribed antimicrobials based on AWaRe classification was calculated. Then the knowledge and practices of prescribing doctor about the utility of AWaRe was determined. The appropriateness of AWaRe classification with days of therapy and defined daily doses of antimicrobial utilization was also estimated.

Statistical analysis

Data was collected on predefined proforma, google form, and excel sheet (Microsoft excel spreadsheet software, office 2016) and analysed by estimating the proportion of various variables including antimicrobial utilizations as per AWaRe

classification. Univariate analysis was performed and the data was arranged as percentages of total. Mean daily dosing for a particular antimicrobial was determined and compared with WHO DDD using the Students *T* test. *P* value less than 0.05 was considered statistical significant.

RESULTS

The research was carried out in accordance with the methodology presented in Figure 1. A total of *n* = 123 patients were enrolled in this study, with each of them receiving antibiotic prescriptions. The majority of these prescriptions were issued to inpatients (75.4%), and both the Medicine and Surgical departments were equally represented, accounting for 49.6% and 50.4%, respectively. Among the healthcare providers responsible for prescribing antibiotics, 72% were Junior Residents, 18.7% were Senior Residents, and 9.3% were Consultants. These findings have been summarized in Table 1.

The prescriptions included 27 different antibiotics, with metronidazole being the most prescribed (19%) followed by ceftriaxone (17%). The mean number of antibiotics used per patient was 1.84 ± 0.83. The mean duration of antibiotics prescribed was 6.63 ± 3.83 days. The maximum number of antibiotics prescribed per patient was five. According to the AWaRe classification, 57.61% of antibiotics fell under the Access, 38.27% in Watch, and 4.11% in Reserve categories, suggesting appropriate antibiotic selection according to these criteria. The distribution of antibiotics prescribed according to the WHO AWaRe categories is presented in Figure 2. The difference in prescribing frequencies amongst departments can be noted. Most of the antibiotics prescribed in the Access category were from the Medicine department (75.4%), followed by Surgery (24.6%). For Watch antibiotics, Medicine had a higher proportion (63.4%) compared to Surgery (36.6%). In terms of seniority, Junior Residents prescribed the highest number of antibiotics for both Access and Watch categories in Medicine and Surgery departments. Senior residents and Consultants prescribed a lower number of antibiotics in all categories and departments. Only a few antibiotics were prescribed in the Reserve category, with most prescriptions being from the Medicine department.

The study also evaluated the Knowledge and Awareness of Healthcare professionals towards the WHO AWaRe classification through a questionnaire survey. A total of 93 participants responded to the survey. Among them, most participants were Junior Residents (69.9%), followed by Senior Residents (25.8%) and Faculty (4.3%). When enquired if they knew about the WHO AWaRe classification only 33.3% of the participants responded positively. Of those who were aware of the AWaRe classification, the most common source of information was the internet (31.2%), followed by the antimicrobial policy of their institution (15.1%) as seen in Table 2.

The survey results on the knowledge and awareness of AMR among healthcare professionals are also presented in Tables 3 and 4. Out of the 93 participants, 68 (73.1%) agreed that the emergence of AMR is inevitable, while only 13 (14.0%) disagreed that AWaRe usage will result in the inability to treat serious infections. Additionally, 58 (62.4%) agreed that it will lead to lengthier hospital stays, 43 (46.2%) agreed that the success of chemotherapy and major surgery will be hampered, and the majority also agreed that its use will lead to increased cost of treatment and increased mortality rates. Regarding the utilization of AWaRe in the hospital summarized in Tables 4 and 5, 35.5% of the participants agreed that it should be used, while only 2.2% disagreed. Additionally, 34.4% agreed that AWaRe reduces adverse effects of inappropriate prescription. However, 37.6% of the participants considered that AWaRe threatens a clinician's autonomy and 30.1% thought that its use can delay treatment.

Additionally, the DDD of each drug was also evaluated. The usage of various antimicrobial drugs in a hospital setting, along with their daily doses and DDD according to the WHO's Anatomical Therapeutic Chemical classification system was calculated. Some of the important findings include high usage rates of ceftriaxone and metronidazole, and relatively low usage rates of drugs like colistin and clindamycin. Additionally, some drugs had wider ranges than others. Comparison of WHO defined DDD with Daily Drug dose (Mean) in the studied prescriptions is represented in the Clustered Bar chart in Figure 3.

Finally, the Mean Daily Drug Dose for prescribed drugs was compared with WHO defined DDD for each drug using a Student's *T* test. The mean daily drug dose of amoxy/clav was significantly higher than the WHO DDD (1.8 *vs* 1.50, $P = 0.014$), while the mean daily drug dose of metronidazole and doxycycline were significantly lower than the WHO DDD ($P < 0.001$ and $P = 0.008$, respectively). The mean daily drug dose of piperacillin/tazobactam, amikacin, clindamycin, and levofloxacin did not show significant differences compared to the WHO DDD ($P > 0.05$).

DISCUSSION

The findings of this study highlight several important issues related to antimicrobial prescribing practices and awareness among healthcare professionals. This study, conducted in a tertiary care institute in North India included prescriptions having at least one antibiotic in both inpatient (75.4%) and outpatient (24.6%) settings collected over a duration of 2 months by universal sampling. A total of 123 patient prescriptions were included which included 243 individual antibiotics. The mean number of antibiotics used per patient was 1.84 ± 0.83 which coincides with a study conducted in Brazil[9] with a mean of 2.4 antibiotics per patient. This finding is in line with the WHO prescribing indicators which state that each prescription should contain an average of 1.6–1.8 antibiotics[7]. The use of multiple antibiotics can increase the risk of adverse effects, drug interactions, and development of AMR. The mean duration of antibiotics prescribed was 6.63 ± 3.83 d. A study conducted in a tertiary care hospital in India reported a mean duration of 5.7 ± 3.1 d[12], while another in Ethiopia reported a mean duration of 10.2 d[13].These variations could be attributed to differences in patient demographics, disease prevalence, prescribing habits, and hospital policies.

Table 1 Descriptive data representing the antibiotic usage patterns categorized according to the designation of prescriber, department, outpatient *vs* inpatient and AWaRe classification

Category	Counts	% of total
Total number of patients	123	
Total prescriptions	123	
No. of different antibiotics used	27	
Most common antibiotic	Metronidazole	19
Designation		
Senior resident	42	18.7
Junior resident	162	72.0
Consultant	21	9.3
OPD/IPD		
OPD	55	24.6
IPD	169	75.4
Department		
Medicine	112	49.6
Surgery	114	50.4
WHO AWaRe classification		
Access	140	57.61
Watch	93	38.27
Reserve	10	4.11
	Antibiotics used in a patient	**Duration of antibiotics**
Mean	1.84	6.63
Median	2	6.00
Standard deviation	0.833	3.83
Minimum	1	1.00
Maximum	5	19.0

Second half of the table represents the descriptive analysis of antibiotics used and duration of individual antibiotics. OPD: Outpatient; IPD: Inpatient; WHO: World Health Organization.

In the present study, prescriptions included 27 different antibiotics of which Metronidazole was the most prescribed antibiotic, which could be due to its activity against anaerobic bacteria, which are commonly implicated in infections of the gastrointestinal tract, genitourinary tract, and skin. It is prescribed mostly in combination with amoxicillin in obstetrics and gynaecological care (mainly in post-delivery prophylaxis), and occasionally in caesarean section. Similar results were found study at Ghana Police Hospital[14]. This was followed by Ceftriaxone, a third-generation cephalosporin from the Watch group. The high proportion of cephalosporins in the prescriptions is consistent with the WHO analysis of South-East Asian countries which also found a very high level of consumption of the same in all states in India [15].

The WHO AWaRe classification system was used to analyse the antibiotics prescribed in this study, and the results showed that most antibiotics fell under the Access (57.61%) category. These antibiotics have a narrow spectrum of activity, lower cost, a good safety profile and generally low resistance potential and should be widely available and affordable. While this is a positive finding, it is also important to note that a significant proportion of antibiotics prescribed were from the Watch category (38.27%), which includes antibiotics that are at risk of becoming ineffective due to overuse and misuse. The small percentage of antibiotics prescribed in the Reserve category (4.11%) is reassuring, as these antibiotics are reserved for use as a last resort and should only be used in highly specific circumstances. The overall prescribing of antibiotics in our study is lower than the WHO recommendations, which state that more than 60% of all prescribed antibiotics must be from the Access group by 2023. Figures in our study are consistent with another Indian study which had Access (53.31%), Watch (40.09%) from, and Reserve (3.40%) category[16], and study done in Zambia with (n = 384) which had Access (55.5%), Watch (43.1%) and Reserve (1.4%)[7]. Our findings differ from another study in India[17] in which Watch group antibiotics accounted for 53.19 % of the total antibiotics and a Bangladesh study in which

Table 2 Representation of knowledge of World Health Organization Access, Watch, Reserve classification among healthcare professionals

Category	Counts	% of total
Position		
Junior resident	65	69.9
Senior resident	24	25.8
Faculty	4	4.3
Do you know about WHO AWaRe classification		
No details	22	23.7
Yes	31	33.3
Never heard	21	22.6
Little Idea	19	20.4
How did you hear about AWaRe?		
The internet	29	31.2
The WHO website	10	10.8
The antimicrobial policy of our institution	14	15.1
Other sources	19	20.4
No idea about it	21	22.6

AWaRe: Access, Watch, Reserve; WHO: World Health Organization.

Table 3 Knowledge (Score) on World Health Organization Access, Watch, Reserve classification

	Total score	Junior resident (*n* = 21)	Senior resident (*n* = 9)	Consultant (*n* = 3)	Medicine (*n* = 24)	Surgery (*n* = 9)
n	33	-	-	-	-	-
Mean	3.91	3.81	4.22	3.67	3.79	4.22
Standard deviation	2.17	2.25	2.33	1.53	2.08	2.49
Minimum	1	1	1	2	1	1
Maximum	8	8	8	5	8	8

WHO: World Health Organization.

64.0% of the patients were treated with antibiotics from the Watch group, 35.6% were treated with antibiotics from the Access group, and only 0.1% were treated with antibiotics from the Reserve group. The higher proportion of Access category antibiotics prescribed in our study could be attributed to the fact that these antibiotics are commonly used for the treatment of common infections encountered in the hospital, such as urinary tract infections, respiratory tract infections, and skin and soft tissue infections. The low proportion of Reserve category antibiotics prescribed in our study is consistent with the recommended sparing use of these antibiotics by WHO and other guidelines, which recommend their use only as a last resort in the treatment of severe infections[18]. In terms of departments and designations, our study found that the Medicine department prescribed the majority of antibiotics in both the Access and Watch categories, followed by the Surgery department. This finding is consistent with previous studies conducted in India and other low- and middle-income countries, where the Medicine department was found to be the highest prescriber of antibiotics[19].

To assess knowledge and attitude of prescribing doctor about the utility of AWaRe a Questionnaire based survey was carried out. Although (73.1%) agreed that the emergence of AMR is inevitable the lack of awareness about the programmes and measures being taken to curb it is concerning. In contrast to this study, a study conducted in the United States found that healthcare professionals had a high level of knowledge about AMR and the appropriate use of antibiotics[20]. This difference could be attributed to variations in healthcare systems and education programs in different countries. Moreover, in a study conducted in Germany, the majority of physicians agreed that the rational use of antibiotics is important for the prevention of AMR[21].

Despite the WHO AWaRe classification being included in the antibiotic policy of our institution only 33.3% of the responders knew about it which shines a light on the gap between the measures being taken and their implementation.

Table 4 Representation of awareness towards World Health Organization Access, Watch, Reserve classification among healthcare professionals

Statement	False	True	No idea	*n*
Emergence of Antimicrobial Resistance is inevitable	24	68	1	93
It will result in inability to treat serious infections	13	80	0	93
Lengthier hospital stays will be a result	35	58	0	93
Success of chemotherapy and major surgery will be hampered	50	43	0	93
Cost of treatment will be increased	52	41	0	93
MMR and IMR will increase	62	31	0	93

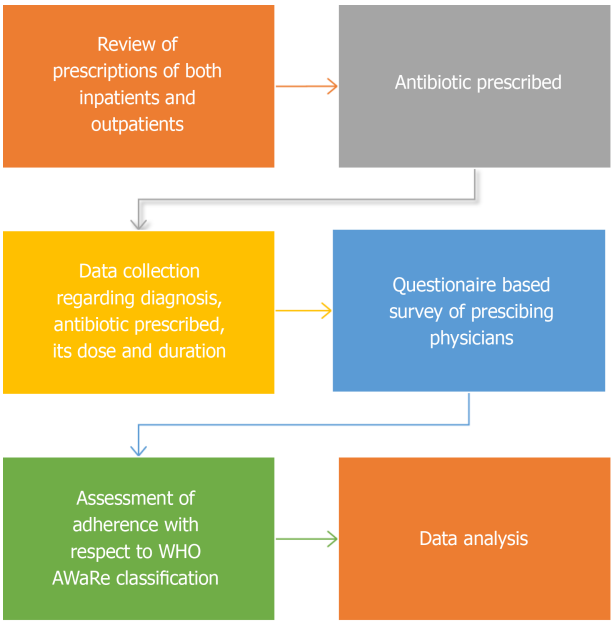
AWaRe: Access, Watch, Reserve; WHO: World Health Organization; MMR: Maternal mortality rate; IMR: Infant Mortality Rate

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Table 5 Representation of attitude towards World Health Organization Access, Watch, Reserve classification among healthcare professionals

Question	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
Should AWaRe be used in the hospital?	18 (19.4%)	2 (2.2%)	21 (22.6%)	33 (35.5%)	19 (20.4%)
AWaRe reduces adverse effects of inappropriate prescription	12 (12.9%)	5 (5.4%)	25 (26.9%)	32 (34.4%)	19 (20.4%)
AWaRe threatens a clinician's autonomy	9 (9.7%)	35 (37.6%)	35 (37.6%)	11 (11.8%)	3 (3.2%)
It can delay treatment	16 (17.2%)	37 (39.8%)	28 (30.1%)	8 (8.6%)	4 (4.3%)

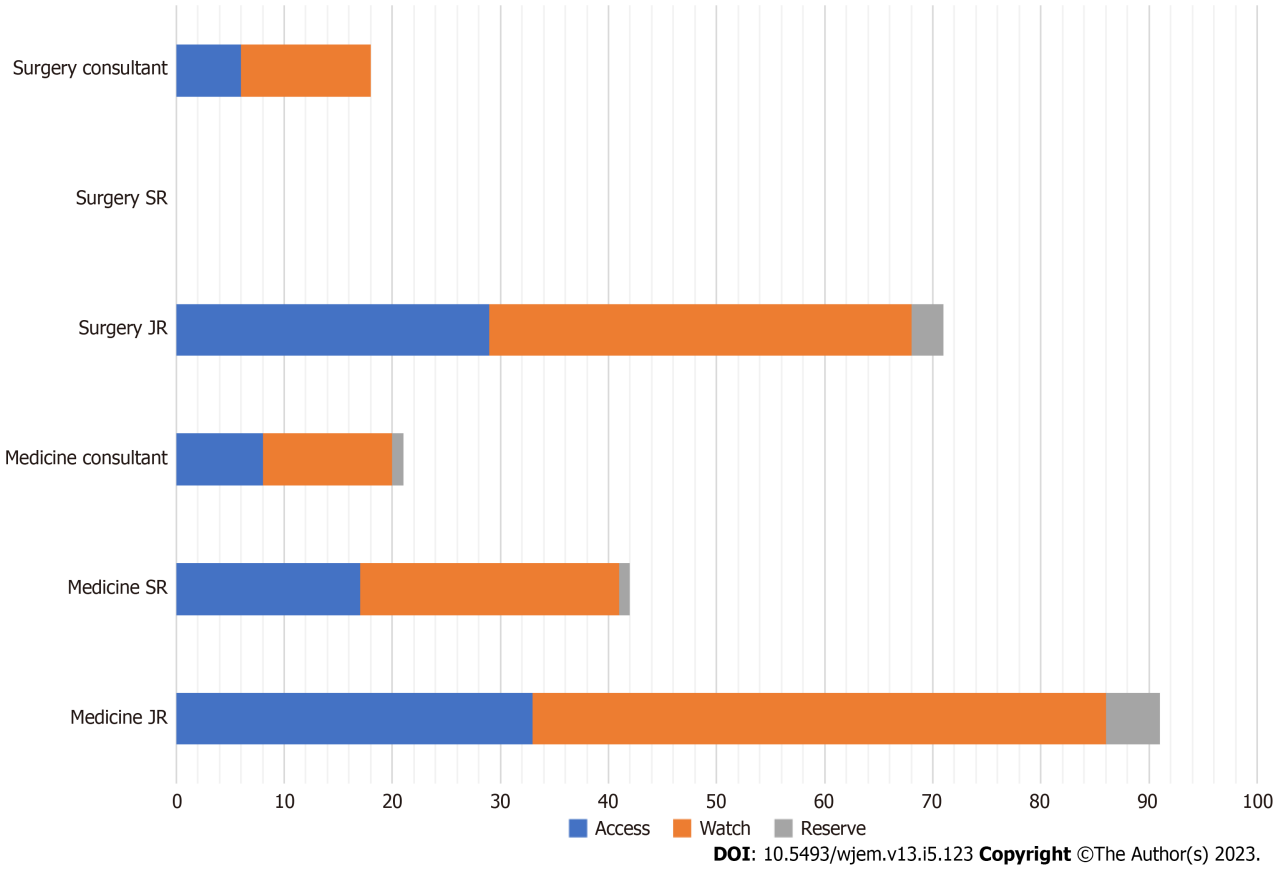
AWaRe: Access, Watch, Reserve.



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Figure 1 Representation of the study flow. WHO: World Health Organization.

The institute antibiotic policy authorizes only the Senior Residents and Consultants to prescribe antibiotics from Watch and Reserve groups but in this study Junior Residents prescribed the highest number of antibiotics for both Access and Watch categories which coincides with previous studies conducted in India and other countries. In a study conducted in a tertiary care hospital in India, junior residents prescribed the majority (68.7%) of antibiotics[19]. The higher proportion of antibiotics prescribed by Junior Residents in our study could be attributed to their relatively higher workload and less clinical experience compared to Senior Residents and Consultants. Overall, the findings of this study suggest that there is



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Figure 2 Frequencies of World Health Organization Access, Watch, Reserve category by designation and department.

a moderate level of awareness among healthcare professionals about AMR, there is a need for further education and awareness programs to ensure the appropriate use of antibiotics in the hospital setting. It must be noted that most of the responders of the survey were also Junior Residents and hence education programmes can hence play a vital role in improving the results by working at grassroot levels.

Another analysis of the study depicts that there is a significant difference between the WHO DDD and the prescribed daily dose in the analysed prescriptions. Several studies conducted in the West have compared the mean daily drug dose of prescribed drugs with the WHO defined DDD. In a study conducted in a hospital in Italy found that the mean daily dose of antibiotics was generally lower than the WHO DDD for most antibiotics[22]. These findings are similar to the present study, which found higher mean daily doses of amoxicillin/clavulanic acid and piperacillin/tazobactam, and a lower mean daily dose of levofloxacin. The WHO DDD is a standardized measure of drug consumption used to compare the drug consumption between different countries and regions. It represents the assumed average maintenance dose per day for a drug used for its main indication in adults. On the other hand, the prescribed daily dose refers to the actual dose that a physician prescribes to a patient.

The difference between the WHO DDD and the prescribed daily dose can have several implications. Firstly, it can lead to overuse or underuse of medications, which can affect the therapeutic outcomes of patients. For example, if the prescribed daily dose is lower than the WHO DDD, the patient may not receive the optimal therapeutic effect of the medication. Conversely, if the prescribed daily dose is higher than the WHO DDD, the patient may be at risk of adverse effects or toxicity. Secondly, the difference between the WHO DDD and the prescribed daily dose can affect the comparability of drug consumption data between different regions and countries. If different regions or countries use different prescribed daily doses, it can be difficult to compare their drug consumption patterns. Therefore, adherence to the WHO DDD is important to ensure that drug consumption data are standardized and comparable across different regions and countries. In conclusion, the difference between the WHO DDD and the prescribed daily dose is an important issue that needs to be addressed in the prescribing practices of physicians. Adherence to the WHO DDD can help to ensure optimal therapeutic outcomes for patients and comparability of drug consumption data between different regions and countries.

Overall, the findings of this study emphasize the importance of improving antimicrobial prescribing practices and increasing awareness among healthcare professionals regarding the WHO AWaRe classification system and the threat of AMR. Effective antimicrobial stewardship programs that promote appropriate antibiotic use can help reduce the risk of AMR and improve patient outcomes. Future research should focus on implementing such programs in hospital settings and evaluating their effectiveness in reducing inappropriate prescribing practices.

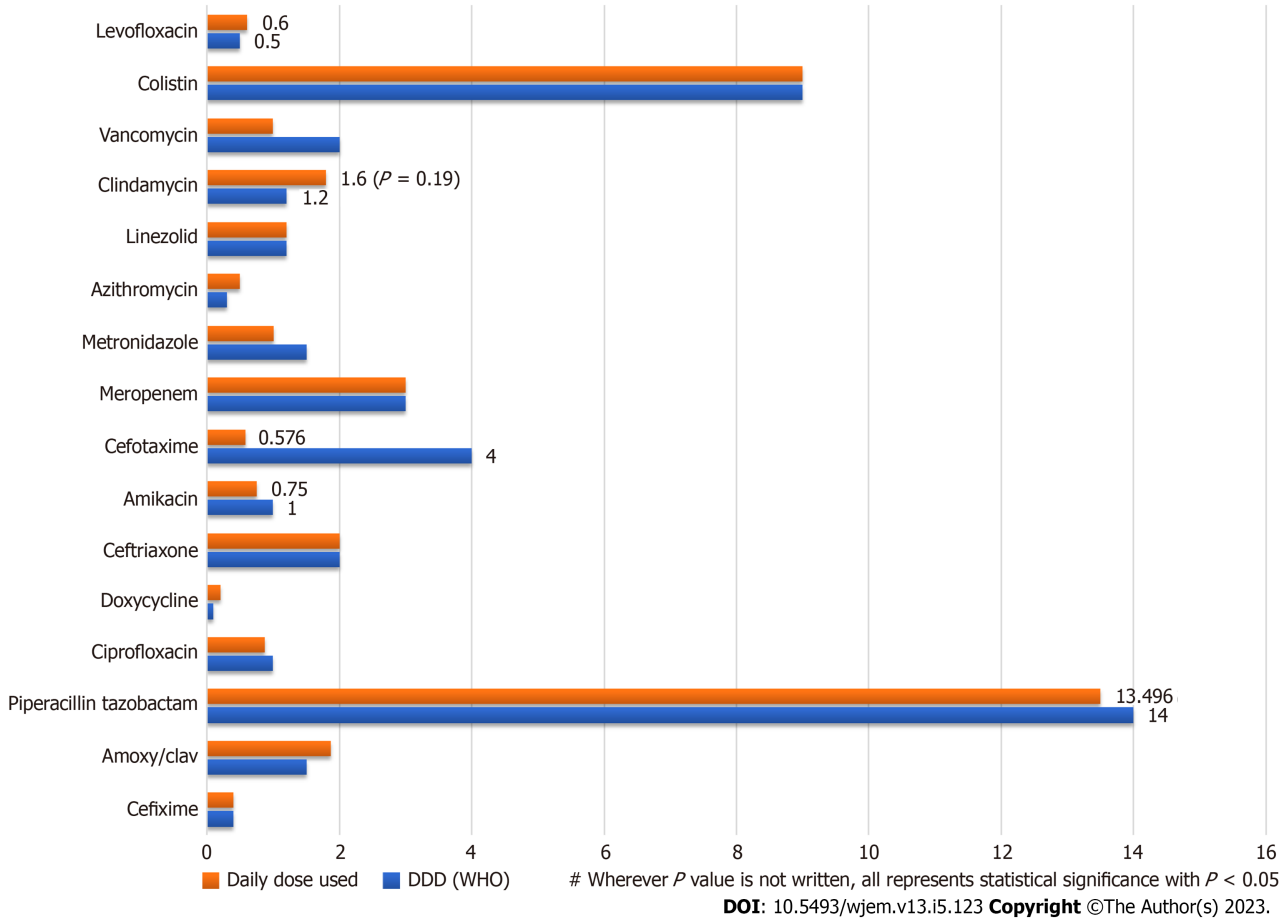


Figure 3 Comparison of drug utilization to World Health Organization defined daily doses.

CONCLUSION

This research indicates an appropriate proportion of prescriptions falling under the Access category (57.61%), suggesting appropriate antibiotic selection, a significant proportion also belongs to the Watch category (38.27%), emphasizing the need for greater caution to prevent the escalation of AMR. There is a moderate level of awareness among healthcare professionals about AMR and the steps being taken to tackle it, highlighting the gap in implementation of policies and need for more steps to be taken in spreading the knowledge about the subject. However, there is a significant difference between the WHO DDD and the prescribed daily dose in the analysed prescriptions suggesting overuse and underuse of antibiotics.

ARTICLE HIGHLIGHTS

Research background

India has particularly high rates of antimicrobial resistance (AMR), posing a threat to effective treatment. The World Health Organization (WHO) Access, Watch, Reserve classification system was introduced to address this issue and guide appropriate antibiotic prescribing. However, there is a lack of studies examining the prescribing patterns of antimicrobials using the AWaRe classification, especially in North India.

Research motivation

This study aimed to assess the prescribing patterns of antimicrobials using the WHO AWaRe classification in a tertiary care centre in North India.

Research objectives

(1) To audit the prescribing patterns of antimicrobials among clinicians using WHO's AWaRe classification in a tertiary care centre; (2) To assess knowledge and practices of prescribing doctor about the utility of AWaRe by Questionnaire based assessment; and (3) To compare the appropriateness of AWaRe classification with days of therapy and defined daily doses of antimicrobial utilization.

Research methods

A descriptive, cross-sectional study was conducted from July 2022 to August 2022 at a tertiary care hospital. Prescriptions containing at least one antimicrobial were included in the study. A questionnaire-based survey was also conducted to assess the knowledge and practices of prescribing doctors regarding the utility of AWaRe classification.

Research results

The study involved a total of 123 patients, each of whom received at least one antimicrobial prescription. Most prescriptions were for inpatients, metronidazole and ceftriaxone were the most prescribed antibiotics. According to the AWaRe classification, 57.61% of antibiotics fell under the Access category, 38.27% in Watch, and 4.11% in Reserve. The questionnaire survey showed that only a third of participants were aware of the AWaRe classification, and there was a lack of knowledge regarding AMR and the potential impact of AWaRe usage.

Research conclusions

This study highlights the need for better antimicrobial prescribing practices and increased awareness of the WHO AWaRe classification and AMR among healthcare professionals.

Research perspectives

The findings indicate a high proportion of prescriptions falling under the Access category, suggesting appropriate antibiotic selection. There is room for improvement and educational interventions and antimicrobial stewardship programs should be implemented to enhance knowledge and adherence to guidelines, ultimately contributing to the containment of AMR.

FOOTNOTES

Author contributions: Negi G, KB A, and Panda PK gave the concept, designed the project, collected the data, analysed, wrote the manuscript, critically reviewed, and approved the manuscript.

Institutional review board statement: The study was reviewed and approved by Institutional Ethics Committee of All India Institute of Medical Sciences, Rishikesh, India.

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Country/Territory of origin: India

ORCID number: Gunjita Negi 0009-0002-3542-9061; Arjun KB 0000-0003-4621-7996; Prasan Kumar Panda 0000-0002-3008-7245.

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REFERENCES

1 WHO Antibiotics Portal [Internet] [DOI: 10.1093/ww/9780199540884.013.u230558]

2 **Amaha ND**, Weldemariam DG, Berhe YH. Antibiotic consumption study in two hospitals in Asmara from 2014 to 2018 using WHO's defined daily dose (DDD) methodology. *PLoS One* 2020; **15**: e0233275 [PMID: 32614832 DOI: 10.1371/journal.pone.0233275]

3 **World Health Assembly 49**. The world health report 1996: fighting disease, fostering development [Internet]. World Health Organization; 1996 [DOI: 10.2307/2137484]

4 One Health Trust [Internet]. [DOI: 10.4060/ca7442en]

5 WHO report on surveillance of antibiotic consumption [Internet] [DOI: 10.1211/pj.2018.20205732]

6 **Godman B**, Egwuenu A, Haque M, Malande OO, Schellack N, Kumar S, Saleem Z, Sneddon J, Hoxha I, Islam S, Mwita J, do Nascimento

RCRM, Dias Godói IP, Niba LL, Amu AA, Acolatse J, Incoom R, Sefah IA, Opanga S, Kurdi A, Chikowe I, Khuluza F, Kibuule D, Ogunleye OO, Olalekan A, Markovic-Pekovic V, Meyer JC, Alfadl A, Phuong TNT, Kalungia AC, Campbell S, Pisana A, Wale J, Seaton RA. Strategies to Improve Antimicrobial Utilization with a Special Focus on Developing Countries. *Life (Basel)* 2021; **11** [PMID: 34200116 DOI: 10.3390/life11060528]

7 **Mudenda S**, Nsofu E, Chisha P, Daka V, Chabalenge B, Mufwambi W, Kainga H, Kanaan MHG, Mfune RL, Mwaba F, Zulu M, Tembo R, Mwasinga W, Chishimba K, Mwikuma G, Monde N, Samutela M, Chiyangi HK, Mohamed S, Matafwali SK. Prescribing Patterns of Antibiotics According to the WHO AWaRe Classification during the COVID-19 Pandemic at a Teaching Hospital in Lusaka, Zambia: Implications for Strengthening of Antimicrobial Stewardship Programmes. *Pharmacoepidemiology* 2023; **2**: 42-53 [DOI: 10.3390/pharma2010005]

8 **Amponsah OKO**, Buabeng KO, Owusu-Ofori A, Ayisi-Boateng NK, Hämeen-Anttila K, Enlund H. Point prevalence survey of antibiotic consumption across three hospitals in Ghana. *JAC Antimicrob Resist* 2021; **3**: dlab008 [PMID: 34223086 DOI: 10.1093/jacamr/dlab008]

9 **Fonseca LG**, de Oliveira Conterno L. Audit of antibiotic use in a Brazilian University Hospital. *Braz J Infect Dis* 2004; **8**: 272-280 [PMID: 15565257 DOI: 10.1590/s1413-86702004000400002]

10 **Gajdács M**, Ábrók M, Lázár A, Burián K. Urinary Tract Infections in Elderly Patients: A 10-Year Study on Their Epidemiology and Antibiotic Resistance Based on the WHO Access, Watch, Reserve (AWaRe) Classification. *Antibiotics (Basel)* 2021; **10** [PMID: 34572680 DOI: 10.3390/antibiotics10091098]

11 AWaRe [Internet]. [cited May 23, 2023]. Home. Available from: https://adoptaware.org/

12 **Ahmad A**, Khan MU, Malik S, Mohanta GP, Parimalakrishnan S, Patel I, Dhingra S. Prescription patterns and appropriateness of antibiotics in the management of cough/cold and diarrhea in a rural tertiary care teaching hospital. *J Pharm Bioallied Sci* 2016; **8**: 335-340 [PMID: 28216959 DOI: 10.4103/0975-7406.199340]

13 **Demoz GT**, Kasahun GG, Hagazy K, Woldu G, Wahdey S, Tadesse DB, Niriayo YL. Prescribing Pattern of Antibiotics Using WHO Prescribing Indicators Among Inpatients in Ethiopia: A Need for Antibiotic Stewardship Program. *Infect Drug Resist* 2020; **13**: 2783-2794 [PMID: 32884305 DOI: 10.2147/IDR.S262104]

14 **Darkwah TO**, Afriyie DK, Sneddon J, Cockburn A, Opare-Addo MNA, Tagoe B, Amponsah SK. Assessment of prescribing patterns of antibiotics using National Treatment Guidelines and World Health Organization prescribing indicators at the Ghana Police Hospital: a pilot study. *Pan Afr Med J* 2021; **39**: 222 [PMID: 34630834 DOI: 10.11604/pamj.2021.39.222.29569]

15 **Das N**, Madhavan J, Selvi A, Das D. An overview of cephalosporin antibiotics as emerging contaminants: a serious environmental concern. *3 Biotech* 2019; **9**: 231 [PMID: 31139546 DOI: 10.1007/s13205-019-1766-9]

16 **Bansal A**, Sharma R, Prakash R. Adoption of the World Health Organization access, watch reserve index to evaluate and monitor the use of antibiotics at a tertiary care hospital in India. *Perspect Clin Res* 2022; **13**: 90-93 [PMID: 35573458 DOI: 10.4103/picr.PICR_202_19]

17 **Mugada V**, Mahato V, Andhavaram D, Vajhala SM. Evaluation of Prescribing Patterns of Antibiotics Using Selected Indicators for Antimicrobial Use in Hospitals and the Access, Watch, Reserve (AWaRe) Classification by the World Health Organization. *Turk J Pharm Sci* 2021; **18**: 282-288 [PMID: 34157817 DOI: 10.4274/tjps.galenos.2020.11456]

18 Access, watch, reserve: How a key policy tool can accelerate the fight against antimicrobial resistance [Internet]. World Health Organization; [cited Oct 12, 2023]. Available from: https://www.who.int/southeastasia/news/opinion-editorials/detail/access-watch-reserve-how-a-key-policy-tool-can-accelerate-the-fight-against-antimicrobial-resistance

19 **Patel A**, Naagar M, Singh R. A study on prescribing pattern of antibiotics in medicine ward of tertiary care teaching hospital. *Int J Basic Clin Pharmacol* 2020; **9**: 887-890 [DOI: 10.18203/2319-2003.ijbcp20202176]

20 **Owens MR**, Brito-Silva F, Kirkland T, Moore CE, Davis KE, Patterson MA, Miketinas DC, Tucker WJ. Prevalence and Social Determinants of Food Insecurity among College Students during the COVID-19 Pandemic. *Nutrients* 2020; **12** [PMID: 32825251 DOI: 10.3390/nu12092515]

21 **Kraus S**, Schiavone F, Pluzhnikova A, Invernizzi AC. Digital transformation in healthcare: Analyzing the current state-of-research. *J Bus Res* 2021; **123**: 557-567 [DOI: 10.1016/j.jbusres.2020.10.030]

22 **Giacomelli A**, Ridolfo AL, Oreni L, Vimercati S, Albrecht M, Cattaneo D, Rimoldi SG, Rizzardini G, Galli M, Antinori S. Consumption of antibiotics at an Italian university hospital during the early months of the COVID-19 pandemic: Were all antibiotic prescriptions appropriate? *Pharmacol Res* 2021; **164**: 105403 [PMID: 33359914 DOI: 10.1016/j.phrs.2020.105403]

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ORIGINAL ARTICLE

Basic Study

In vitro study on the transmission of multidrug-resistant bacteria from textiles to pig skin

Pavlina Lena, Spyridon Karageorgos, Maria Liatsou, Aris P Agouridis, Nikolaos Spernovasilis, Demetris Lamnisos, Panagiotis Papageorgis, Constantinos Tsioutis

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Pavlina Lena, Mpn Unilab Clinical Laboratory, Nicosia 1066, Cyprus

Pavlina Lena, Maria Liatsou, Demetris Lamnisos, Department of Health Sciences, School of Sciences, European University Cyprus, Nicosia 2404, Cyprus

Spyridon Karageorgos, First Department of Pediatrics, "Aghia Sophia" Children's Hospital, National and Kapodistrian University of Athens, Athens 11527, Greece

Aris P Agouridis, Constantinos Tsioutis, School of Medicine, European University Cyprus, Nicosia 2404, Cyprus

Aris P Agouridis, Nikolaos Spernovasilis, Infectious Diseases, German Oncology Center, Limassol 4108, Cyprus

Panagiotis Papageorgis, Department of Life Sciences, School of Sciences, European University Cyprus, Nicosia 2404, Cyprus

Corresponding author: Nikolaos Spernovasilis, BSc, MD, MSc, PhD, Director, Infectious Diseases, German Oncology Center, Nikis 1, Limassol 4108, Cyprus.
nikolaos.spernovasilis@goc.com.cy

Abstract

BACKGROUND

The survival of microorganisms on textiles and specifically on healthcare professionals' (HCP) attire has been demonstrated in several studies. The ability of microorganisms to adhere and remain on textiles for up to hours or days raises questions as to their possible role in transmission from textile to skin *via* HCP to patients.

AIM

To evaluate the presence, survival and transmission of different multidrug-resistant bacteria (MDRB) from HCP attire onto skin.

METHODS

Three MDRB [methicillin-resistant *Staphylococcus aureus* (MRSA); vancomycin-resistant *Enterococcus faecium* (VRE); carbapenem-resistant *Klebsiella pneumoniae*, CRKP)] were inoculated on textiles from scrubs (60% cotton-40% polyester) and

white coat (100% cotton) at concentrations of 10^8 colony-forming units (CFU), 10^5 CFU, and 10^3 CFU per mL. The inoculation of swatches was divided in time intervals of 1 min, 5 min, 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h. At the end of each period, textiles were imprinted onto pig skins and each skin square was inverted onto three different selective chromogenic media. Growth from the pig skin squares was recorded for the 3 MDRB at the three above concentrations, for the whole length of the 6-h experiment.

RESULTS

MRSA was recovered from pig skins at all concentrations for the whole duration of the 6-h study. VRE was recovered from the concentration of 10^8 CFU/mL for 6 h and from 10^5 CFU/mL for up to 3 h, while showing no growth at 10^3 CFU/mL. CRKP was recovered from 10^8 CFU/mL for 6 h, up to 30 min from 10^5 CFU/mL and for 1 min from the concentration of 10^3 CFU/mL.

CONCLUSION

Evidence from the current study shows that MRSA can persist on textiles and transmit to skin for 6 h even at low concentrations. The fact that all MDRB can be sustained and transferred to skin even at lower concentrations, supports that textiles are implicated as vectors of bacterial spread.

Key Words: Textiles; Attire; Multidrug-resistant bacteria; Methicillin-resistant *Staphylococcus aureus*; Vancomycin-resistant *Enterococcus faecium*; Extended-spectrum b-lactamase; Pig skin; Skin; Transmission

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Core Tip: The current study aimed to evaluate the transmission of multidrug-resistant bacteria (MDRB) from attire (scrubs, white coats) onto skin. Three MDRB types [methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium*, carbapenem-resistant *Klebsiella pneumoniae*] were inoculated on textiles over various time intervals for up to 6 h and then imprinted onto pig skin. All MDRB were able to be sustained and transferred to skin, but at different concentrations and time. MRSA had the longest presence on textile and highest transmission potential even at lower concentrations. Evidence suggests that textiles can be implicated as vectors of MDRB spread in the healthcare setting.

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INTRODUCTION

It is well established that textiles can carry micro-organisms, a fact with raises concerns for their ability to transmit them either onto skin or to other textiles[1]. Previous studies and systematic reviews have demonstrated the ability of pathogens, including multidrug-resistant bacteria (MDRB), to survive on healthcare professionals' (HCP) attire, devices and surfaces in hospitals and long-term care facilities, thus raising the need to study their potential to contribute to transmission[1-4].

Cyprus is considered a high-prevalence country for MDRB. In fact, in healthcare-associated infections in Cyprus hospitals in 2020, methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA) was detected in 49.1% of invasive infections by *S. aureus*, vancomycin-resistant *Enterococcus faecium* (*E. faecium*) (VRE) accounted for 44.1% of enterococci, and isolates of *Klebsiella pneumoniae* (*K. pneumoniae*) exhibited multidrug resistance in 18.2% and carbapenem resistance in 19.8%[5]. Further to this, in a previous study, our group demonstrated the presence of several different MDRB on HCP uniforms in hospitals and long-term care facilities in Cyprus, including MRSA, VRE, extended-spectrum b-lactamase (ESBL)-producing bacteria and carbapenem-resistant bacteria[1]. These findings suggest that MDRB may be transmitted in different ways within healthcare settings, thus raising the need to identify additional areas for targeted interventions and improvement.

A demonstrable means to evaluate the role of attire and textiles in the transmission cycle, is by studying the degree of transmission of micro-organisms from fabrics to skin. To achieve this, it is necessary to use an alternative to human skin with similar properties, such as pig skin, in order to allow accurate *in-vitro* experimentation[6]. The similarities between human and pig skin lie in the structure of epidermis and thickness ratios between dermis and epidermis, as well as in hair follicle and blood vessel patterns. In contrast to the skin of other animals used in laboratory studies, the dermal collagen and elastic content of the pig skin are more similar to the skin of humans. Furthermore, Schmook *et al*[7] demonstrated in an *in-vitro* study of percutaneous absorption, that in the absence of human tissue, pig skin was the most suitable model.

Based on current literature, including experimental and observational studies, we hypothesize that MDRB can carry the ability to survive on textiles to a considerable extent to be isolated and to transmit onto skin. Our aim was to evaluate the survival potential of different MDRB on different types of textiles and to evaluate their potential for transmission onto skin, using representative strains such as MRSA, VRE and carbapenem-resistant *K. pneumoniae* (CRKP).

MATERIALS AND METHODS

Aims

The aim of the current study was to determine the viability of MDRB over a 6-h period, which mimics the usual duration of a hospital shift, on two types of textiles and to evaluate whether these inoculated textiles can colonize pig skin using three MDRB (MRSA, VRE and CRKP) at different concentrations. Study approval was not required as it did not involve human subjects or animals. The strains were retrieved from a previous study that was approved by the Cyprus National Bioethics Committee (decision number "ΕΕΒΚ ΕΠ 2018.01.155").

Materials

Fresh MRSA, VRE and CRKP cultures were grown from previously isolated organisms from HCP uniforms from a previous study, that were stored in -20 °C. After 24 h of incubation, the organisms were prepared at 0.5 MacFarland standard each. Their precise concentrations were calculated: 1.18×10^8 CFU/mL MRSA, 1.6×10^8 CFU/mL for *E. faecium*, and 1.14×10^8 CFU/mL for *K. pneumoniae*. All 3 organisms were serially diluted from 10^8 CFU down to 10^2 CFU. The concentrations of 10^8, 10^5, and 10^3 CFU per mL were used during the process.

Textile swatches were cut in squares of 1.5 cm × 1.5 cm and sterilised in 100 °C in dry oven for 2 h. The swatches were taken from uniforms and scrubs used in healthcare settings in order to mimic txextiles used in real-life conditions, with a composition of 60% cotton and 40% polyester (T1, taken from a used scrub uniform of a nurse, who had worn it for 6 mo), and 100% cotton (T2, taken from a white coat used by a physician for 6 mo). Aluminium foil squares of 1.5 cm × 1.5 cm were cut and heat sterilised in the same manner as the cloth swathes. These would be used as a barrier when pressure would be applied on cloth or skin.

Fresh pig skin (commercially retrieved 24 h after slaughtering), cleared from most of the fat under the dermis, was scrubbed and washed with chlorhexidine soap and then with alcohol. The cleaned skin was then dried in examination paper and kept covered to avoid drying. When the experiment was ready to begin, square pieces of 1.5 cm × 1.5 cm of skin were cut using sterilized blades. Negative control skin samples were incubated on the 3 chromogenic media to ensure the absence of the 3 microorganisms used. The specialized chromogenic media used were CHROMO agar, MRSA medium, chromo VRE, and chromo ESBL.

Experimental design

The current experimental study was designed in duration and environmental conditions that mimic the working conditions within a 6-h HCP shift. Temperature of the laboratory room was kept constant at 22.9-23.5 °C and humidity of 49%-53% and the experiment was conducted on the bench.

Each microbial concentration had 10 stations allocated at time intervals of 1 min, 5 min, 15 min, 30 min, 1 h, and then every hour for a total of 6 h and at three different concentrations. Each station had in place: 2 cloth swatches (T1 & T2), 2 respective skin squares (S1 & S2) and chromogenic plates, for each of the 3 dilutions of the microorganisms (Figure 1).

Firstly, 2 cloth swatches in each station were inoculated with 50 μL of each dilution in each column. The inoculation started from 6 h up to 1 min, guided by stopwatches. At the end of each time slot, each cloth swatch was placed on top of the skin square. The cloth was applied onto skin with a pressure of 100 Gr (friction asset) for 5 s and foil was placed over it for protection. Afterwards and following the same technique, each skin square was pressed onto the chromogenic agar. The plates were then incubated at 37 °C. After 24 h, the incubated plates were checked and incubated for an additional 24 h. When the 48-h incubation period ended, the chromogenic plates were inspected, and the presence of growth was recorded. We did not perform a concentration count at each station; the results indicated presence or not. Thus, the experiment was performed based on the assumption that even a small amount of bacteria could be transmitted and possibly cause infection.

RESULTS

Over a period of 6 h and at different time points, a total of 3 MDRB (MRSA, VRE, CRKP) were inoculated in 3 different concentrations (10^8 CFU/mL, 10^5 CFU/mL, 10^3 CFU/mL) on 2 different cloth textiles (T1, T2) and then each cloth was applied on a pig skin square (S1, S2 respectively). The skin inoculates were then cultured on special medium for 48 h and MDRB growth was recorded.

All findings demonstrating the recovery of the 3 MDRB from skin and textiles are presented in Table 1. Recovery times for all 3 MDRB were the same in both skin swatches S1 and S2 that were in contact with T1 (60% cotton and 40% polyester) and T2 (100% cotton), respectively. Overall, no differences were observed in terms of growth for each MDRB between the two types of textiles. MRSA exhibited the highest recovery. In specific, recovery of MRSA was successful at all time intervals and for all 3 concentrations (10^8 CFU/mL, 10^5 CFU/mL and 10^3 CFU/mL). MRSA remained detectable and could be transmitted throughout the 6-h experiment duration. VRE was recovered from the highest concentration of

Table 1 Growth indicated in skin swatches S1 from textile T1 (cotton-polyester cloth) and S2 from T2 (100% cotton cloth), when imprinted on chromogenic agars at three concentrations

Time	MRSA (CFU/mL)						VRE (CFU/mL)						CRKP (CFU/mL)					
	S1 (10^8)	S2 (10^8)	S1 (10^5)	S2 (10^5)	S1 (10^3)	S2 (10^3)	S1 (10^8)	S2 (10^8)	S1 (10^5)	S2 (10^5)	S1 (10^3)	S2 (10^3)	S1 (10^8)	S2 (10^8)	S1 (10^5)	S2 (10^5)	S1 (10^3)	S2 (10^3)
1 min	G	G	G	G	G	G	G	G	G	G	/	/	G	G	G	G	G	G
5 min	G	G	G	G	G	G	G	G	G	G	/	/	G	G	G	G	/	/
15 min	G	G	G	G	G	G	G	G	G	G	/	/	G	G	G	G	/	/
30 min	G	G	G	G	G	G	G	G	G	G	/	/	G	G	G	G	/	/
60 min	G	G	G	G	G	G	G	G	G	G	/	/	G	G	/	/	/	/
2 h	G	G	G	G	G	G	G	G	G	G	/	/	G	G	/	/	/	/
3 h	G	G	G	G	G	G	G	G	G	G	/	/	G	G	/	/	/	/
4 h	G	G	G	G	G	G	G	G	/	/	/	/	G	G	/	/	/	/
5 h	G	G	G	G	G	G	G	G	/	/	/	/	G	G	/	/	/	/
6 h	G	G	G	G	G	G	G	G	/	/	/	/	G	G	/	/	/	/

CFU: Colony-forming units; CRKP: Carbapenem-resistant *Klebsiella pneumoniae*; G: Growth; MRSA: Methicillin-resistant *Staphylococcus aureus;* VRE: Vancomycin-resistant *Enterococcus faecium*.

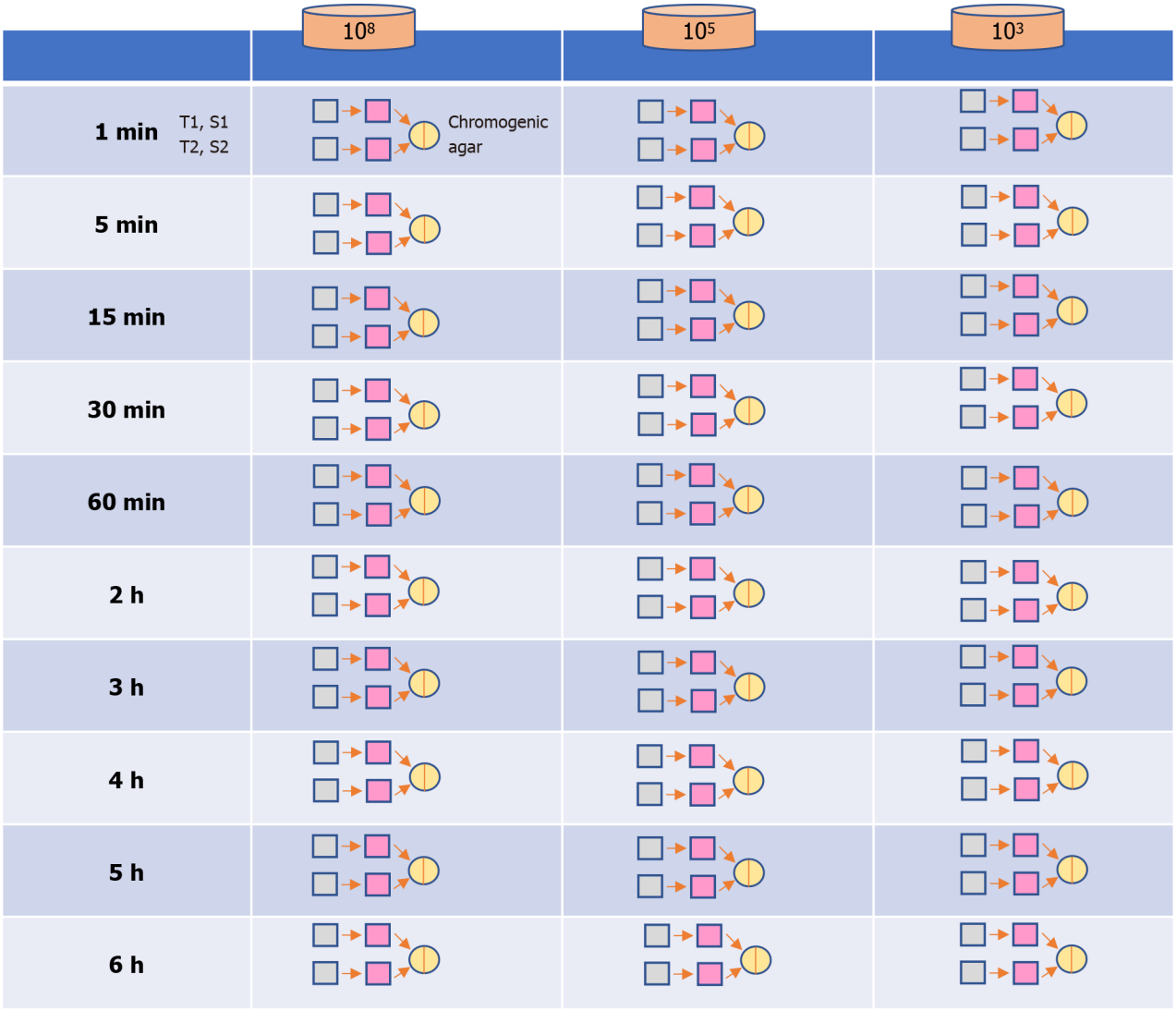
10^8 CFU/mL for the whole duration of the 6-h period and for up to 3 h from the 10^5 CFU/mL concentration. No recovery of VRE was recorded from the lowest concentration of 10^3 CFU/mL. CRKP was also recovered from the highest concentration of 10^8 CFU/mL for the total duration of 6 h (duration of the experiment) and for 30 min from the second highest concentration (10^5 CFU/mL), whereas it was recovered for only up to 1 min from the lowest concentration of 10^3 CFU/mL.

Conclusively, our results support the sustainability of MRSA for the maximum of the duration of the study in all concentrations. The other gram-positive coccus (VRE) also remained for the whole duration of the study but only in the highest concentration, whereas VRE was not isolated for the lowest concentration. In contrast, the gram-negative bacterium (CRKP) remained for less time in the concentration of 10^5 CFU/mL and for only 1 min at the lowest concentration.

DISCUSSION

In our study, involving the transfer and presence of MDRB as a result of contact between textiles and pig skin, MRSA exhibited the longest persistence out of the 3 studied MDRB and over the duration of 6 h of the experiment. VRE and CRKP were both detected at the highest concentration of 10^5 CFU/mL, for up to 3 h and 30 min, respectively. The presence of MDRB, as recorded on both textile types that were used in the study, was also confirmed on pig skin, which provides evidence of potential for transfer of bacteria and MDRB onto skin from contaminated textiles and furthermore, suggesting this as a transmission mode to patients. The study results show that gram-positive cocci, such as staphylococci and enterococci, are more resilient on textiles and in the environment over time, whereas CRKP also showed prolonged presence at higher concentrations.

To our knowledge, only few previous studies have evaluated the transferability of bacteria onto skin. Butler *et al*[8] conducted a study on the transfer of bacteria onto pigskin by use of white coats. Specifically, MRSA, VRE and *Acinetobacter baumannii* (*A. baumannii*) (reported as pan-resistant) were inoculated on cloth swatches and rubbed on sanitized pigskin. All 3 MDRB exhibited ability to transfer from cloth to pig skin at time intervals of 1, 5 and 30 min, whereas *A. baumannii* was transferred up to a dilution of 1:1000. Desai *et al*[9] also confirmed sustainability and transmission of MRSA from cotton towels and bedsheets to pig skin for long periods reaching up to 14 d, whereas Sattar *et al*[10] reported transfer of MRSA from textiles (cotton and polycotton) to other textiles and to finger skin. The transfer was fivefold higher when the textile was moist and when there was friction. Varshney *et al*[11] in a study transfer between textiles of *Acinetobacter calcoaceticus, Escherichia coli* (*E. coli*) and *S. aureus,* also reported that cell transfer varied between 5%-61% when friction was applied compared to non-friction, which suggested the importance of the role of rubbing between same textiles. Malnick *et al*[12] examined the transfer of bacteria between fabric and surrogate skin taking into consideration the effect of surface energy and surface roughness of fabrics, while using *E. coli* and *S. aureus* against 100% cotton, 100% polyester and a 50-50 blend of both. Their conclusion was that the 100% polyester attracted the highest number of bacteria.



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Figure 1 Experimental set up: Each station contains 2 cloth swatches (T1 & T2), 2 pig skins (S1 & S2), and one chromogenic agar.

The survival ability of microorganisms on textiles has been previously addressed by several studies. Koca *et al*[13] used different fabrics, such as cotton, cotton-polyester, silk, and wool. To assess persistence of a variety micro-organisms, including yeasts, fungi, MRSA, VRE and ESBL-producing bacteria. Results showed that VRE had its longest survival on cotton-polyester fabrics (51 d, and 49 d on 100% cotton, silk and wool). MRSA had the longest survival period of 41 d on wool and 37 d on cotton silk and cotton-polyester. Respectively *E. coli* showed its longest survival for 45 d on cotton, silk and wool, and on cotton-polyester 37 d. These findings were confirmed by Malnick *et al*[12] who showed that 50% of VRE-swabbed pyjamas and bed sheets yielded micro-organisms overnight. Similarly, Neely and Maley[3] studied the survival of strains of MRSA and VRE on five different types of materials used commonly in hospitals: 100% cotton (which represented cloths and towels), cotton-polyester, 100% polyester, and 100% polypropylene plastic (represented which splash aprons). MRSA had the longest survival on polypropylene plastic (> 51 d), followed by 40 d (polyester), and 21 d (cotton). VRE exhibited long survival periods of > 80 d on polypropylene plastic and polyester, and 22 d on the other materials.

In agreement with the above findings, a literature review supported that contaminated textiles can harbour bacteria and consequently transmit infection for weeks[14]. Current evidence supports survival and persistence on textiles not only of bacteria, but also mycobacteria, fungi and viruses. Specifically, VRE can survive for more than 90 d, MRSA for up to 56 d and *K. pneumoniae* for 11 d. Survival at room temperature favoured polyester against cotton. Even though there is only a limited number of reported healthcare-associated infection outbreaks associated with contaminated textiles[15-19], their involvement may be undermined, and not sought out during incidences or as part of the general infection control process.

Taken cumulatively, the findings of our study add insight to the literature and clearly suggest that staff attire, including uniforms and scrubs, when contaminated, can transmit pathogenic micro-organisms onto patients' skin, thus acting as bacterial vectors. It can also be assumed that contaminated hands can in turn contaminate uniforms[10]. Hence, strict and targeted preventive measures targeting textile and attire are needed to break the chain of transmission. Current evidence from various studies suggests against the use of white coats and ties in healthcare settings, as they are more

frequently contaminated compared to other HCP attire[4]. On the other hand, short-sleeved uniforms can be more beneficial as regards to lower transmission of pathogenic micro-organisms[20]. However, these measures alone seem insufficient to control MDRB spread and support the need for additional control measures, such as ensuring application of appropriate home laundering practices, use of a hospital laundry service, wearing single-use protective aprons or gowns wherever applicable, promoting hand hygiene before and after patient interaction, daily uniform change, and application of contact precautions particularly in high-prevalence settings[21,22]. Our experimental study was performed under the same environmental conditions for all 3 studied MDRB. Therefore, the different observations in regard to time intervals and concentrations among the 3 studied MDRB in our study, probably reflect the properties of these bacterial strains.

The strength of the current study lies in its design, which aimed to resemble as much as possible a real-life situation. Specifically, in addition to evaluating the possibility of textile inoculation and transfer onto pig skin, we confirmed the viability of clinically important MDRB over time, in an attempt to mimic the environmental conditions in healthcare settings during HCP shifts. Our findings demonstrate that 6 h after MDRB inoculation of textiles, transfer from a dry textile surface on pig skin was possible. Simulation of real-life conditions showed that MDRB can survive, grow and transmit from textiles of two different compositions onto pig skin, which is the closest parallel to human skin.

Limitations of our study include the small spectrum of micro-organisms used and the fact that we used only two types of fabrics. We also acknowledge the fact that during our experiment, we didn't quantify the bacterial colony concentrations after inoculation. However, we performed the experiment under the hypothesis that bacterial persistence through time and transmission is evidence that textiles can be infectious, regardless of the growth amount. In addressing the current research gaps in the literature and the limitations of our study, future studies can include inoculation of textiles not solely with pure bacterial cultures, but also mixed with organic matter such as bodily fluids, to better emulate real-life conditions. Furthermore, the role of skin flora could be investigated in the survival of microorganisms on textiles and to evaluate their effect on MDRB binding capacity and replication.

Of note, only few similar studies have attempted to associate textiles with the transmission and survival of microorganisms. In line with the general perception that the risk of transmission from textiles is low, there is not enough emphasis given to the importance of cleaning and decontamination of textiles, compared to other inanimate surfaces[22,23]. However, our findings are supportive that textiles can be responsible for the transmission of pathogens in healthcare settings and thus, they should be managed accordingly. Moreover, our study strongly suggests that textiles should be included in the transmission triad "patient - healthcare professional - environment". To this end, healthcare settings should opt to analyse all possible steps in the chain of transmission and introduce appropriate action plans that include reduction of transmission risk through textiles and HCP attire. In the absence of protocols for testing uniform cleanliness and compliance, our findings provide evidence towards enforcing appropriate measures to reduce bacterial reservoirs in healthcare settings.

CONCLUSION

In conclusion, the current experimental study using 3 types of MDRB, provides evidence of their sustainability and transmission from textiles to skin. MRSA exhibited maximum sustainability for the whole duration of the 6-h study in all concentrations, VRE also remained for the whole duration of the study but only in the highest concentration, whereas CRKP remained for less time overall. Cumulatively, our data adds support to increasing evidence that textiles should be considered as vehicles of transmission in the healthcare setting.

ARTICLE HIGHLIGHTS

Research background

The isolation of microorganisms from textiles, including healthcare professionals' (HCP) attire, has been previously demonstrated in several studies.

Research motivation

The ability of microorganisms to adhere and survive on textiles, raises questions as to their possible role in transmission from textile to skin in healthcare environments.

Research objectives

The present experimental study aimed to evaluate the presence, survival and transmission of different multidrug-resistant bacteria (MDRB) from HCP attire onto skin.

Research methods

Inoculation of 3 MDRB [methicillin-resistant *Staphylococcus aureus* (MRSA); vancomycin-resistant *Enterococcus faecium* (VRE); carbapenem-resistant *Klebsiella pneumoniae*, CRKP)] on textiles from two types of textiles (60% cotton-40% polyester and 100% cotton) at 3 different concentrations (10^8 CFU, 10^5 CFU and 10^3 CFU per mL) and at different time intervals ranging from 1 min to 6 h. At the end of each time period, textiles were imprinted onto pig skins and each skin

square was inverted onto selective chromogenic media. Growth from the pig skins was recorded for the 3 MDRB at the three above concentrations, for the whole length of the 6-h experiment.

Research results

Recovery of MDRB from pig skins differed for each strain, with MRSA recording the longest and most sustained recovery at all concentrations and for up to 6 h. VRE showed no growth from 10^3 CFU/mL and was recovered from 10^8 CFU/mL for 6 h and from at 10^5 CFU/mL for up to 3 h. CRKP was recovered from 10^8 CFU/mL for 6 h, up to 30 min from 10^5 CFU/mL and for only 1 min from 10^3 CFU/mL.

Research conclusions

Evidence from the current study shows that all 3 studied MDRB can be sustained and transferred onto skin, with MRSA showing the highest level of persistence on textiles and transmission to skin even at low concentrations.

Research perspectives

Our findings support that textiles can be implicated as vectors of bacterial spread.

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FOOTNOTES

Author contributions: Lena P contributed to the conceptualization of this study; Lena P, Liatsou M, Lamnisos D, Papageorgis P, and Tsioutis C involved in the methodology of the manuscript; Lena P, Karageorgos S, Agouridis AP, Spernovasilis N, Lamnisos D, Papageorgis P, and Tsioutis C participated in the formal analysis and investigation of this manuscript; Lena P, Karageorgos S, and Tsioutis C drafted the manuscript; Lena P, Karageorgos S, Agouridis AP, Spernovasilis N, and Tsioutis C contributed to the review and editing of this manuscript; Lena P, Papageorgis P, and Tsioutis C involved in the supervision of this study; and all authors approved final article version published.

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Data sharing statement: No additional data to those presented in the study are available.

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Country/Territory of origin: Cyprus

ORCID number: Nikolaos Spernovasilis 0000-0002-6981-8535; Constantinos Tsioutis 0000-0002-7865-8529.

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REFERENCES

1 **Lena P**, Karageorgos SA, Loutsiou P, Poupazi A, Lamnisos D, Papageorgis P, Tsioutis C. Multidrug-Resistant Bacteria on Healthcare Workers' Uniforms in Hospitals and Long-Term Care Facilities in Cyprus. *Antibiotics (Basel)* 2021; **11** [PMID: 35052926 DOI: 10.3390/antibiotics11010049]
2 **Lena P**, Ishak A, Karageorgos SA, Tsioutis C. Presence of Methicillin-Resistant Staphylococcus aureus (MRSA) on Healthcare Workers' Attire: A Systematic Review. *Trop Med Infect Dis* 2021; **6** [PMID: 33807299 DOI: 10.3390/tropicalmed6020042]
3 **Neely AN**, Maley MP. Survival of enterococci and staphylococci on hospital fabrics and plastic. *J Clin Microbiol* 2000; **38**: 724-726 [PMID: 10655374 DOI: 10.1128/JCM.38.2.724-726.2000]
4 **Haun N**, Hooper-Lane C, Safdar N. Healthcare Personnel Attire and Devices as Fomites: A Systematic Review. *Infect Control Hosp Epidemiol* 2016; **37**: 1367-1373 [PMID: 27609491 DOI: 10.1017/ice.2016.192]
5 **Eurosurveillance Editorial Team**. ECDC and WHO/Europe joint report on tuberculosis surveillance and monitoring in Europe. *Euro Surveill* 2014; **19** [PMID: 24679724 DOI: 10.2807/ese.19.11.20741-en]
6 **Turaga KK**, Bhagavatula G. What should doctors wear? *BMJ* 2008; **337**: a938 [DOI: 10.1136/bmj.a938]

7 **Schmook FP**, Meingassner JG, Billich A. Comparison of human skin or epidermis models with human and animal skin in in-vitro percutaneous absorption. *Int J Pharm* 2001; **215**: 51-56 [PMID: 11250091 DOI: 10.1016/s0378-5173(00)00665-7]

8 **Butler DL**, Major Y, Bearman G, Edmond MB. Transmission of nosocomial pathogens by white coats: an in-vitro model. *J Hosp Infect* 2010; **75**: 137-138 [PMID: 20299132 DOI: 10.1016/j.jhin.2009.11.024]

9 **Desai R**, Pannaraj PS, Agopian J, Sugar CA, Liu GY, Miller LG. Survival and transmission of community-associated methicillin-resistant Staphylococcus aureus from fomites. *Am J Infect Control* 2011; **39**: 219-225 [PMID: 21458684 DOI: 10.1016/j.ajic.2010.07.005]

10 **Sattar SA**, Springthorpe S, Mani S, Gallant M, Nair RC, Scott E, Kain J. Transfer of bacteria from fabrics to hands and other fabrics: development and application of a quantitative method using Staphylococcus aureus as a model. *J Appl Microbiol* 2001; **90**: 962-970 [PMID: 11412326 DOI: 10.1046/j.1365-2672.2001.01347.x]

11 **Varshney S**, Pandey P, Gupta D, Sharma S. Role of fabric properties, moisture and friction in transfer of bacteria from fabric to fabric. *Text Res J* 2020; **90**: 478-485 [DOI: 10.1177/0040517519866956]

12 **Malnick S**, Bardenstein R, Huszar M, Gabbay J, Borkow G. Pyjamas and sheets as a potential source of nosocomial pathogens. *J Hosp Infect* 2008; **70**: 89-92 [PMID: 18621446 DOI: 10.1016/j.jhin.2008.05.021]

13 **Koca O**, Altoparlak U, Ayyildiz A, Kaynar H. Persistence of nosocomial pathogens on various fabrics. *Eurasian J Med* 2012; **44**: 28-31 [PMID: 25610201 DOI: 10.5152/eajm.2012.06]

14 **Kramer A**, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis* 2006; **6**: 130 [PMID: 16914034 DOI: 10.1186/1471-2334-6-130]

15 **Osawa K**, Baba C, Ishimoto T, Chida T, Okamura N, Miyake S, Yoshizawa Y. Significance of methicillin-resistant Staphylococcus aureus (MRSA) survey in a university teaching hospital. *J Infect Chemother* 2003; **9**: 172-177 [PMID: 12825118 DOI: 10.1007/s10156-002-0225-3]

16 **Barrie D**, Wilson JA, Hoffman PN, Kramer JM. Bacillus cereus meningitis in two neurosurgical patients: an investigation into the source of the organism. *J Infect* 1992; **25**: 291-297 [PMID: 1474265 DOI: 10.1016/0163-4453(92)91579-Z]

17 **Wright SN**, Gerry JS, Busowski MT, Klochko AY, McNulty SG, Brown SA, Sieger BE, Ken Michaels P, Wallace MR. Gordonia bronchialis sternal wound infection in 3 patients following open heart surgery: intraoperative transmission from a healthcare worker. *Infect Control Hosp Epidemiol* 2012; **33**: 1238-1241 [PMID: 23143362 DOI: 10.1086/668441]

18 **Sasahara T**, Hayashi S, Morisawa Y, Sakihama T, Yoshimura A, Hirai Y. Bacillus cereus bacteremia outbreak due to contaminated hospital linens. *Eur J Clin Microbiol Infect Dis* 2011; **30**: 219-226 [PMID: 20938704 DOI: 10.1007/s10096-010-1072-2]

19 **Fijan S**, Turk SŠ. Hospital textiles, are they a possible vehicle for healthcare-associated infections? *Int J Environ Res Public Health* 2012; **9**: 3330-3343 [PMID: 23202690 DOI: 10.3390/ijerph9093330]

20 **Perry C**, Marshall R, Jones E. Bacterial contamination of uniforms. *J Hosp Infect* 2001; **48**: 238-241 [PMID: 11439013 DOI: 10.1053/jhin.2001.0962]

21 **Horner C**, Wilcox M, Barr B, Hall D, Hodgson G, Parnell P, Tompkins D. The longitudinal prevalence of MRSA in care home residents and the effectiveness of improving infection prevention knowledge and practice on colonisation using a stepped wedge study design. *BMJ Open* 2012; **2**: e000423 [PMID: 22240647 DOI: 10.1136/bmjopen-2011-000423]

22 **Owen L**, Laird K. The role of textiles as fomites in the healthcare environment: a review of the infection control risk. *PeerJ* 2020; **8**: e9790 [PMID: 32904371 DOI: 10.7717/peerj.9790]

23 **Loveday HP**, Wilson JA, Hoffman PN, Pratt RJ. Public perception and the social and microbiological significance of uniforms in the prevention and control of healthcare-associated infections: an evidence review. *British J Infect Control* 2007; **8**: 10-21 [DOI: 10.1177/1469044607082078]

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ORIGINAL ARTICLE

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Exploring the mechanism of action bitter melon in the treatment of breast cancer by network pharmacology

Kavan Panchal, Bhavya Nihalani, Utsavi Oza, Aarti Panchal, Bhumi Shah

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Kavan Panchal, Bhavya Nihalani, Utsavi Oza, Aarti Panchal, Bhumi Shah, Pharmaceutical Chemistry, L. J. Institute of Pharmacy, L J University, Gujarat, Ahmedabad 382210, India

Corresponding author: Bhumi Shah, PhD, Associate Professor, Pharmaceutical Chemistry, L. J. Institute of Pharmacy, L J University, Sanand Circle, Sarkhej-Gandhinagar Hwy, Sarkhej, Gujarat, Ahmedabad 382210, India. bhumi197@gmail.com

Abstract

BACKGROUND

Bitter melon has been used to stop the growth of breast cancer (BRCA) cells. However, the underlying mechanism is still unclear.

AIM

To predict the therapeutic effect of bitter melon against BRCA using network pharmacology and to explore the underlying pharmacological mechanisms.

METHODS

The active ingredients of bitter melon and the related protein targets were taken from the Indian Medicinal Plants, Phytochemistry and Therapeutics and SuperPred databases, respectively. The GeneCards database has been searched for BRCA-related targets. Through an intersection of the drug's targets and the disease's objectives, prospective bitter melon anti-BRCA targets were discovered. Gene ontology and kyoto encyclopedia of genes and genomes enrichment analyses were carried out to comprehend the biological roles of the target proteins. The binding relationship between bitter melon's active ingredients and the suggested target proteins was verified using molecular docking techniques.

RESULTS

Three key substances, momordicoside K, kaempferol, and quercetin, were identified as being important in mediating the putative anti-BRCA effects of bitter melon through the active ingredient-anti-BRCA target network study. Heat shock protein 90 AA, proto-oncogene tyrosine-protein kinase, and signal transducer and activator of transcription 3 were found to be the top three proteins in the protein-protein interaction network study. The several pathways implicated in the anti-BRCA strategy for an active component include phosphatidylinositol 3-kinase/protein kinase B signaling, transcriptional dysregulation, axon guidance, calcium signaling, focal adhesion, janus kinase-signal transducer and activator of transcription signaling, cyclic adenosine monophosphate signaling, mammalian

target of rapamycin signaling, and phospholipase D signaling.

CONCLUSION
Overall, the integration of network pharmacology, molecular docking, and functional enrichment analyses shed light on potential mechanisms underlying bitter melon's ability to fight BRCA, implicating active ingredients and protein targets, as well as highlighting the major signaling pathways that may be altered by this natural product for therapeutic benefit.

Key Words: Bitter melon; Momordica charantia; Network pharmacology; Molecular docking; Breast cancer; Indian Medicinal Plants, Phytochemistry and Therapeutics

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Core Tip: The phytochemicals and molecular processes in bitter melon that are thought to be involved in the therapy of breast cancer (BRCA) were investigated using network Pharmacology. Our research demonstrated that the anti-BRCA benefits of bitter melon are likely caused by negative regulation of transcription, cell differentiation, apoptosis, proteolysis, negative control of neuron apoptosis, and cell migration. Further discovered nine important pathways like phosphatidylinositol 3-kinase/protein kinase B signaling, transcriptional dysregulation, axon guidance, calcium signaling, focal adhesion, janus kinase-signal transducer and activator of transcription signaling, cyclic adenosine monophosphate signaling, mammalian target of rapamycin signaling, and phospholipase D signaling, are likely to be involved in the mechanism of action of bitter melon for the treatment of BRCA.

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INTRODUCTION

Breast cancer (BRCA) is the highest-escalation cancer in women with incidence rates rising at a rate of 3% annually in recent years around the world[1]. BRCA is the most prevalent cancer in women, with more than 2.3 million cases diagnosed each year[2]. BRCA is the top or second cause of death from cancer in women in 95% of the world's nations[3]. The age at which BRCA symptoms first appear is also decreasing, which has a severe psychological impact on patients, puts a large amount of financial and life burden on patients and their families, and lowers happiness levels dramatically. BRCA is currently treated with a combination of chemotherapy, radiotherapy, and surgery which have several shortcomings. The psychological and physical side effects include slow wound healing, changes in body shape, liver and kidney damage, changes in metabolism, and severe alopecia[4,5]. As a result, it is important to create medicines that have a wide range of applications and few negative effects. Herbal medicine has a lot of potential because it has few adverse effects and a powerful healing effect. Emergence, expansion, invasion, and metastasis of BRCA are complex processes. Herbal medicine is a useful technique for treating BRCA regardless of stage[6,7].

Bitter melon, also known as momordica charantia, is a member of the Cucurbitaceae family that exhibits anti-inflammatory, potential antibacterial, and antiviral activities[8-10]. bitter melon extracts are also effective against tumors[11] and were also found to be effective for the treatment of ulcers, malaria, pain, psoriasis, hyperlipidemia, and hypertension[12-17]. It includes several different active ingredients, including polysaccharides, anthrone, peptides, proteins, terpenoids, phenolics, and many more. bitter melon anticancer potentials have become more widely known in recent years. According to research, bitter melon suppresses the growth of several malignant tumors, including gastric, liver, and BRCA[18].

According to Ray *et al*[19], the bitter melon extract reduces cell proliferation and induces apoptotic cell death. The fact that bitter melon extract administration increased p53, p21, and pChk1/2 while inhibiting cyclin B1 and cyclin D1 production suggests yet another mechanism involved in controlling the cell cycle. Muhammad *et al*[20] also noted that bitter melon extract treatment enhances phospho-adenosine monophosphate activated protein kinase expression in BRCA cells and suppresses the mammalian target of rapamycin (mTOR)/protein kinase B (Akt) signaling pathway. Additionally, they showed that bitter melon extract feeding effectively slowed the development of BRCA in syngeneic and xenograft animal models. Additionally, they noticed that tumors from animals fed bitter melon extract showed enhanced p62 accumulation, autophagy activation, and apoptotic cell death[20]. Shim *et al*[21] have demonstrated a therapy with bitter melon extract inhibiting the growth of triple-negative BRCA tumors in mouse models more effectively than treatment with estrogen receptor-positive breast tumors. However, the anti-cancer mechanism of bitter melon extract connecting lipid metabolism in BRCA growth is still unknown. They also showed that bitter melon extract feeding reduces acyl-CoA: Cholesterol acyltransferase-1 expression and slows tumor growth in non-obese diabetic scid gamma mice implanted with triple-negative BRCA mammospheres. Their presented study was the first study indicting bitter

melon extract's role in the inhibition of Acyl-CoA: Cholesterol acyltransferase-1 and the proliferation of triple-negative BRCA cells, suggesting it to be beneficial in combination with other therapeutic agents to treat human BRCA[21].

A recently developed analytical technique is network pharmacology. It performs "multitarget and multi-pathway" analysis of pharmaceuticals and diseases through the integration of various databases, and then creates a "drug active ingredient disease" model to visually depict the investigated action mechanism and precisely forecast the targets[22,23]. Even though a significant number of studies have demonstrated bitter melon's clinical effectiveness in the treatment of BRCA, the exact mechanism by which it works is still unknown[24]. This study aimed to identify potential bitter melon components, targets, and biological pathways against BRCA using network pharmacology. Furthermore, we validated and provided a theoretical foundation for the possible mechanism using molecular docking studies (Figure 1).

MATERIALS AND METHODS

Collection and screening of active constituents of bitter melon

Chemical constituents were collected from the Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) 2.0 database (https://cb.imsc.res.in/imppat/)[25]. The bioactive obtained were analyzed and only the chemical constituents of the fruit part were taken by applying the filter. By typing the names of the constituents into the search box on PubChem (https://pubchem.ncbi.nlm.nih.gov/), the canonical smiles of the active constituents were acquired[26]. Moreover, the Molsoft database was used to obtain information regarding the molecular formula, molecular weight, number of hydrogen bond acceptors, number of hydrogen bond donors, MolLogP, and drug-likeness (DL) (https://www.molsoft.com/)[27] to get an idea of whether the compound is following the Lipinski rule. The bioactive violating more than two rules of Lipinski were eliminated. Additionally, the DL and oral bioavailability (OB) thresholds of 0.18 and 30%, respectively, were used to identify the potential active phytochemicals in bitter melon. These two crucial components greatly influence a substance's potential to have pharmacological effects, and from that the final bioactives were selected, and data were prepared and integrated.

Predicting the targets of the compounds

For identification of the potential targets of the bio-actives, the online platform SuperPred was used (https://prediction.charite.de/subpages/target_prediction.php). The targets of the compound were obtained after entering the canonical smiles[28]. The genes of strong binders and predicted targets were taken, and, on the latter, predicted targets with 75% probability were chosen[29]. The gene's names corresponding to the proteins were found in the string by putting all the protein's names, and obtaining the gene UniProt, or official symbol.

Screening for gene targets

The genes related to BRCA were sourced from GeneCards (http://www.genecards.org), using the term "BRCA" as the search query[30]. A Venn diagram was created using the online tool Venny (https://bioinfogp.cnb.csic.es/tools/venny/) to compare the targets of bitter melon and BRCA[31]. The overlap diagram represents the anti-BRCA targets of bitter melon.

Construction and analyses of the protein-protein interactions (PPI) network

The PPI network was constructed by uploading the anti-BRCA targets of bitter melon to the STRING platform (https://string-db.org) with "Homo sapiens" chosen in the organism box[32]. After selecting "Medium Confidence (0.400)" in the basic setting column and "Hide disconnected nodes in the network" in the advanced setting column, the tab-separated values (TSV) format file was exported. The TSV format file was imported into the Cytoscape software to produce the visual analysis of the PPI network[33].

Network construction of active phytoconstituents and anti-BRCA targets

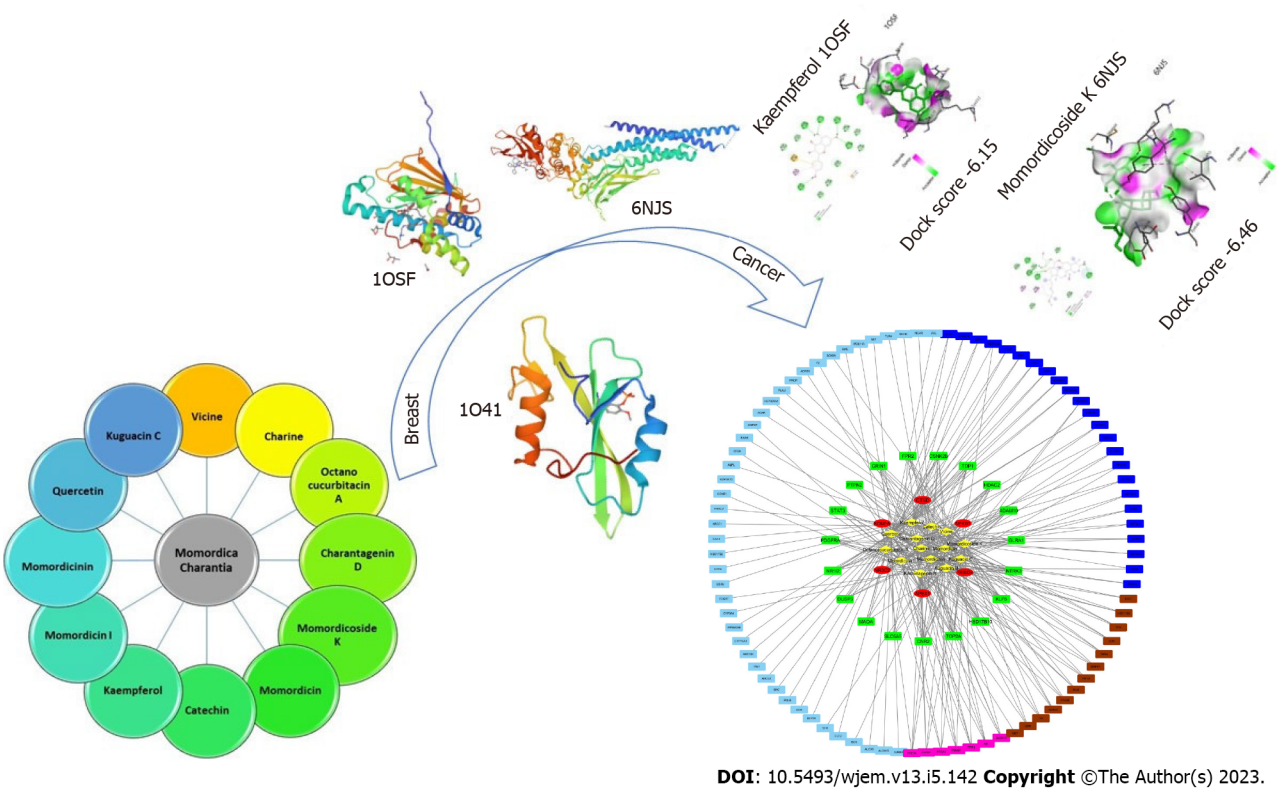
The network of active ingredients in bitter melon and the corresponding targets was constructed and analyzed using the software Cytoscape 3.7.2[34,35]. The nodes in the network represented molecules or genes, which were distinguished by different shapes, and the line between one molecule and one gene meant they were related[36].

Gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis

The biological role of bitter melon's anti-BRCA targets was hypothesized using GO and KEGG pathway enrichment analysis. The GO analysis included molecular function, biological processes, and cellular components[37]. The anti-BRCA targets of bitter melon were uploaded to the DAVID platform (https://david.ncifcrf.gov/) to access the database of GO functions and KEGG pathways[38]. Applying a p value of 0.01 and organising the gene count in descending order allowed the top 10 data from the GO analysis to be sorted. The top 10 pathways were then displayed in the form of an enrichment dot bubble by uploading the data to the Bioinformatics Platform (http://www.bioinformatics.com.cn/)[39].

Verification by molecular docking stimulation

The network pharmacology identified possible bitter melon active components as well as the main BRCA-fighting targets and pathways. The bitter melon active compounds with the highest edge counts and which interact the most with targets were chosen as the ligands. Three bioactives were chosen as the ligands, and the Chem 3D program was used to create their 3D structures after searches in the PubChem database (https://pubchem.ncbi.nlm.nih.gov). After adding



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Figure 1 Graphical abstract.

hydrogens, calculating Gasteiger charges, identifying the root, and selecting rotatable bonds with the help of AutoDock Tools, the ligands were stored in the format of PDBQT. The targets chosen as the protein receptors were those with the top three values in the PPI network, and the 3D structures were taken from the protein data bank (PDB) database (http://www.rcsb.org/)[40]. The specifications of the grid box created around the ligand are shown below.

Hydrogen atoms were added to the receptor structure after the water molecules had been removed, and charges were determined after assigning AD4-type atoms. Thus, prepared receptors were stored in the PDBQT format using the AutoDock tool. All residues remained rigid for the duration of the molecular docking process. The outcomes were shown, and the generation of the binding energy (docking score) was done[40].

RESULTS

Organizing the database of bitter melon

By using the keyword Momordica Charantia in the IMPPAT 2.0 database and reviewing the literature, 304 active ingredients were obtained. By applying a filter from the aerial part of the plant to the fruit part, 43 chemical constituents were obtained, and their canonical smiles were taken to the Molsoft database to demonstrate Lipinski properties and DL. After that, the phytoconstituents that contain DL greater than 0.18, oral OB thresholds of more than 30%, and which do not violate more than 2 Lipinski rules were chosen. So, at last, 14 phytoconstituents' remains were further analyzed for results.

Active phytochemicals targets

The SuperPred database was used to identify possible protein targets for the bitter melon's active phytochemicals. Targeted genes for corresponding compounds were determined by taking strong binders, and 75% filtered probability predicted targets were taken. After redundant information was removed, 133 putative protein targets of bitter melon's active phytochemicals were further examined.

Establishing a database of BRCA

In the GeneCards database, "BRCA" was used as the keyword, and around 15970 genes were obtained. So, to reduce the no. of genes, GeneCards Inferred Functionality Scores filter of > 50 was applied, which resulted in BRCA genes to about 3601.

Acquisition of the venn diagram and construction of the component-target network

A Venn diagram was produced by submitting the bitter melon and BRCA targets to the Venny platform, respectively.

The results conclusively showed that 112 instances of 133 genes connected to bitter melon were likewise linked to BRCA (Figure 2).

Construction and analyses of the PPI network

By inputting 112 anti-BRCA targets into the STRING database and using the Cytoscape program to show them, the PPI network of possible targets was created. There were 428 edges and 112 nodes in the PPI network. The size of map nodes increases as the node degrees rise, and their color shifts from orange to blue. The map edge size increased and the color changed from orange to blue as the total score rose (Figure 3A). On a bar graph, the top 10 genes were displayed in order of their degree (Figure 3B). Each bar was plotted using the degree value associated with each gene. The top 3 potential anti-BRCA targets, *i.e.*, heat shock protein 90 (HSP90) AA1, proto-oncogene tyrosine-protein kinase (SRC), and signal transducer and activator of transcription 3 (STAT3), were chosen for molecular docking studies, with key active phytochemicals described in molecular docking section under result.

Network of active phytoconstituents and anti-BRCA targets

Using the network analysis program Cytoscape, the active ingredient and BRCA target network was created (Figure 4). There are 369 edges and 126 nodes in the network. The 112 circular nodes surrounding the 14 active components of bitter melon, which were represented by the network's yellow diamond nodes in the centre, were anticancer targets. Red was used to indicate 6 target nodes linked to more than 10 active compounds, green for 19 target nodes linked to in the range of 5 to 10 corresponding chemicals, pink for 7 target nodes linked to 4 compounds, brown for 13 target nodes linked to 3 active molecules, dark blue for 24 target nodes linked to 2 compounds, and blue for 43 target nodes linked to only 1 active compound. Amongst all, three phytoconstituents, quercetin, kaempferol, and momordicoside K are considered the most important and key active phytochemicals found in bitter melon for BRCA treatment. The findings show that the majority of bitter melon's active compounds had several targets, and many of these targets related to different active ingredients, demonstrating that bitter melon is a multicomponent, multitarget medication. These outcomes highlight the complex nature of the interactions between many targets and active phytochemicals in bitter melon.

Enrichment analysis

To further analyze the BRCA targets of bitter melon, GO enrichment was performed on 112 intersecting targets using the web-based DAVID program. The top 10 representative enrichment terms for cellular components, molecular processes, and biological processes, sorted by count in descending order and P value < 0.01, are displayed in Figure 5. Orange bars represent a biological process that mainly consisted of G-protein-coupled receptor activity (10/45) and protein kinase binding (9/45). Bars with the green color represented the cellular component, which mainly included the nucleus (42/45), membrane (28/45), and extracellular region (20/45); and lastly, blue colored bars signify the molecular function. Of all 45 potential genes, 9 genes were involved in negative regulation of transcription, DNA template, and cell differentiation.

KEGG pathway enrichment analysis

Using the DAVID web-based tool, KEGG pathway analysis was carried out on 112 overlapping targets to clarify the molecular mechanism by which bitter melon treats BRCA. By uploading 112 anti-BRCA targets to the DAVID platform, around 90 KEGG pathways were obtained, which were further filtered and sorted by P value < 0.01 and gene count in descending order. The top 20 representative enrichment terms were plotted by an enrichment dot bubble in Figure 6. Phosphatidylinositol 3-kinase/protein kinase B (PIK3-Akt) signaling, transcriptional misregulation, axon guidance, calcium signaling, focal adhesion, janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling, cyclic adenosine monophosphate (cAMP) signaling, mTOR signaling, and phospholipase D signaling pathways may play important roles in the mechanism of bitter melon against BRCA. These signaling pathways might work in concert to provide the therapeutic benefits of bitter melon against BRCA.

Molecular docking

HSP90AA, SRC, and STAT3 were employed as the receptors because they have the top three greatest values in the PPI network and the highest level of resolution, while quercetin, kaempferol, and momordicoside k were chosen as the ligands since they each have more than ten gene targets. The PubChem, PDB, Open Babel, and AutoDock tools were used to prepare the ligands and receptors. The three compounds were docked through AutoDock to three different targets to validate the prediction. The result has been displayed as an affinity value (Table 1). Whereas 1O41 had the weakest capacity to attach to the ligands of the three, 1OSF had the easiest time doing so. At a value of -4.47 kcal/mol, quercetin's affinity for 1O41 was the lowest. Figures 7A and B display the docking interactions of kaempferol with 1OSF and 6NJS, respectively, while Figures 8A and B display the docking interactions o momordicoside K with 1OSF and 6NJS, respectively.

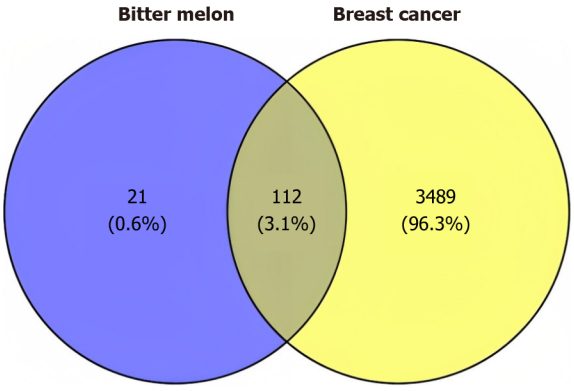
Kaempferol showed many interactions with 1OSF. The hydroxyl group present on the chromen-4-one ring showed hydrogen bonding with Asn106, Lys112, Gly135, and Phe138, while the hydroxyl group present on the phenyl ring showed hydrogen bonding with Asp93. Phenyl ring is also involved in pi-sulfur and pi-alkyl interactions with Met98 and Ala55, respectively. Similarly, the hydroxyl group present on chromen-4-one ring showed hydrogen bonding with Glu638, and Lys658 on 6NJS. The hydroxyl group attached to the phenyl ring showed interactions with Pro639, and Gln644 through hydrogen bonding. Pi-alkyl interactions are observed between chromen-4-one ring and Val637.

Momordicoside K also showed many interactions with 1OSF. Three hydroxyl groups present on the oxane ring form hydrogen bonds with Asn106, Lys112, Gly135, Val136, and Phe138. While the sidechain oxygen atom is involved in hydrogen bond interaction with Gly135. An alkyl interaction is observed between the phenanthrene ring and the alkyl

Table 1 Affinities of compound to the targets (dock score)

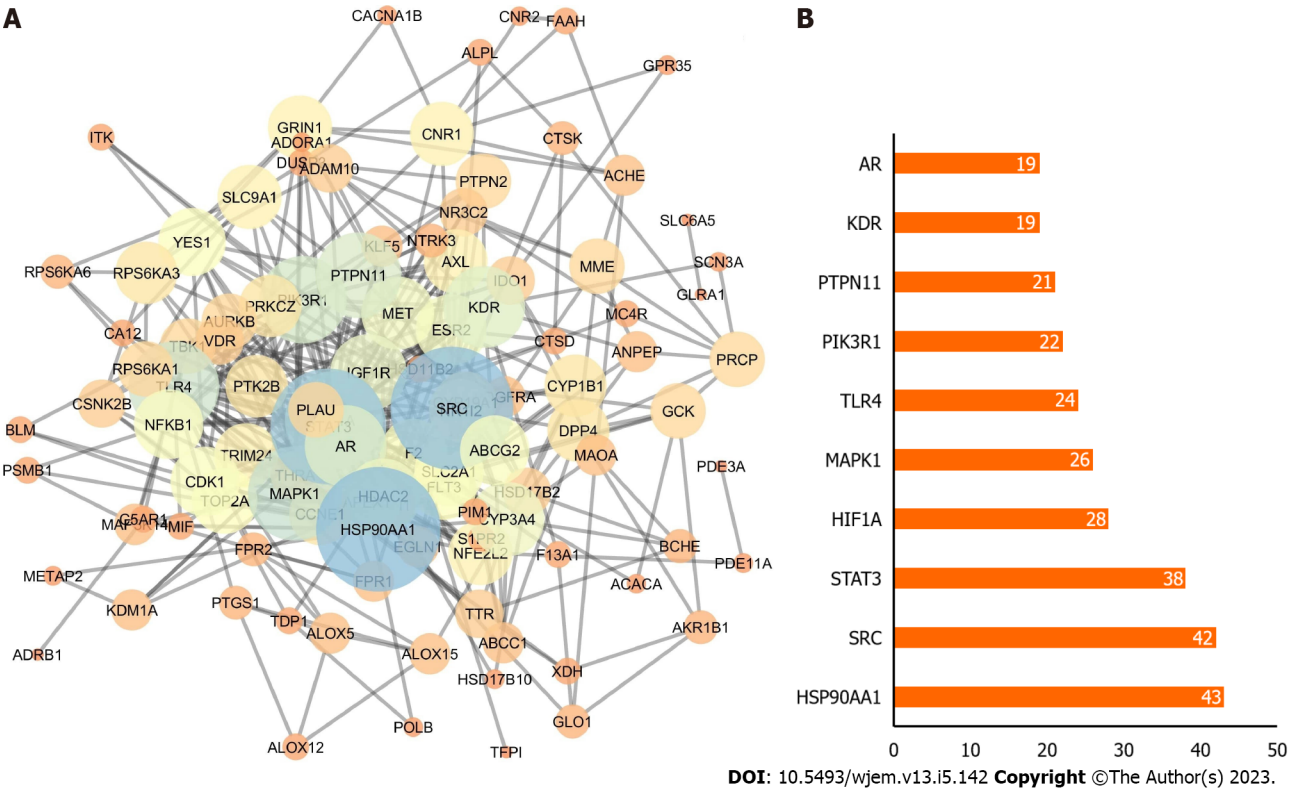
Compound	HSP90AA (1OSF)	SRC (1O41)	STAT3 (6NJS)
Quercetin	-5.57	-4.47	-5.61
Kaempferol	-6.15	-4.74	-5.78
Momordicoside K	-6.07	-5.39	-6.46

HSP90: Heat shock protein 90; SRC: Proto-oncogene tyrosine-protein kinase; STAT3: Signal transducer and activator of transcription 3.



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Figure 2 Venn diagram of targets of bitter melon and breast cancer where blue color indicates targets of bitter melon while yellow color indicates targets of breast cancer.



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Figure 3 Potential targets. A: Protein-protein interaction network; B: Bar chart of the top 10 genes according to degree where X-axis is the degree value, and the Y-axis is the gene name.

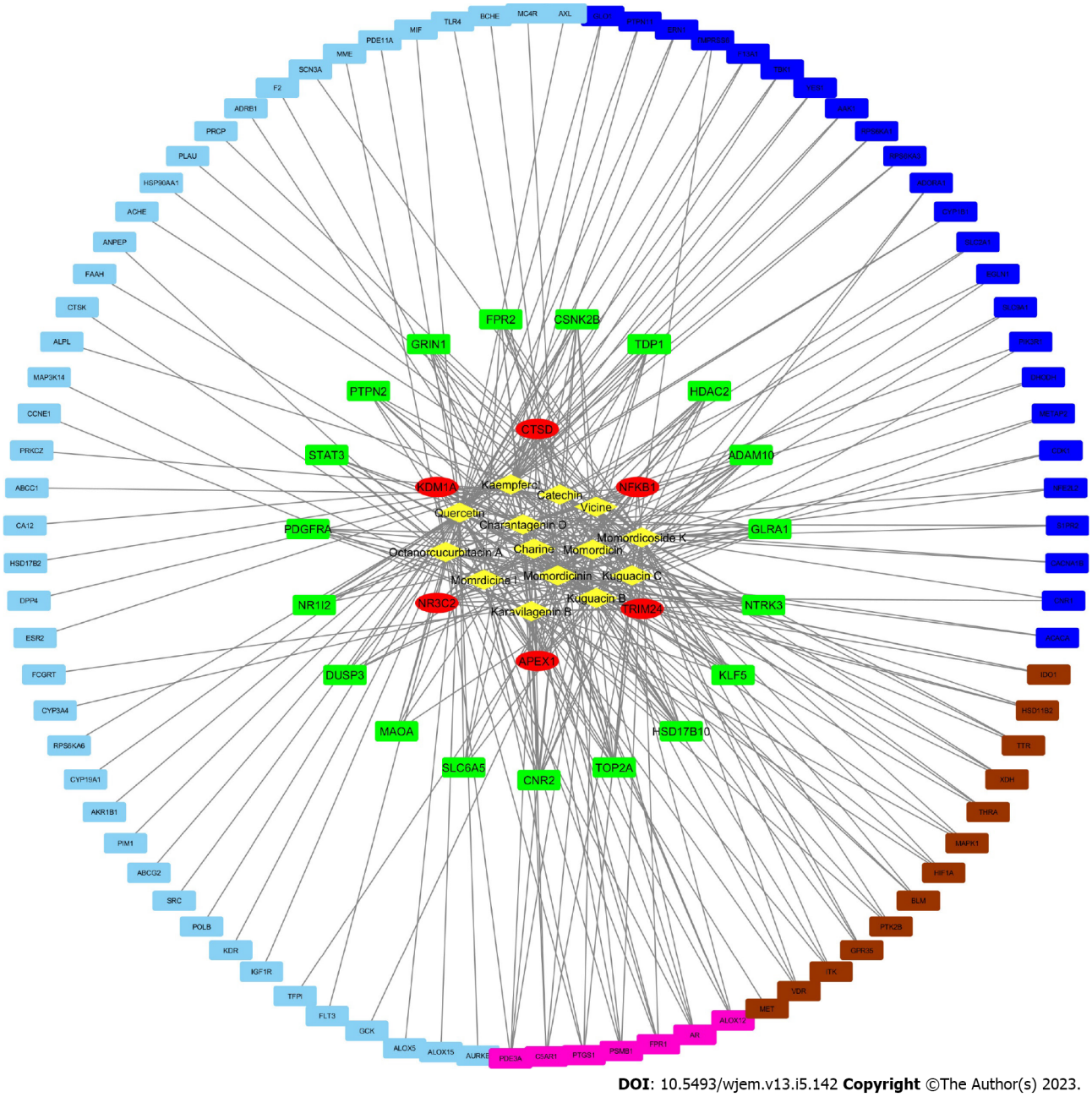
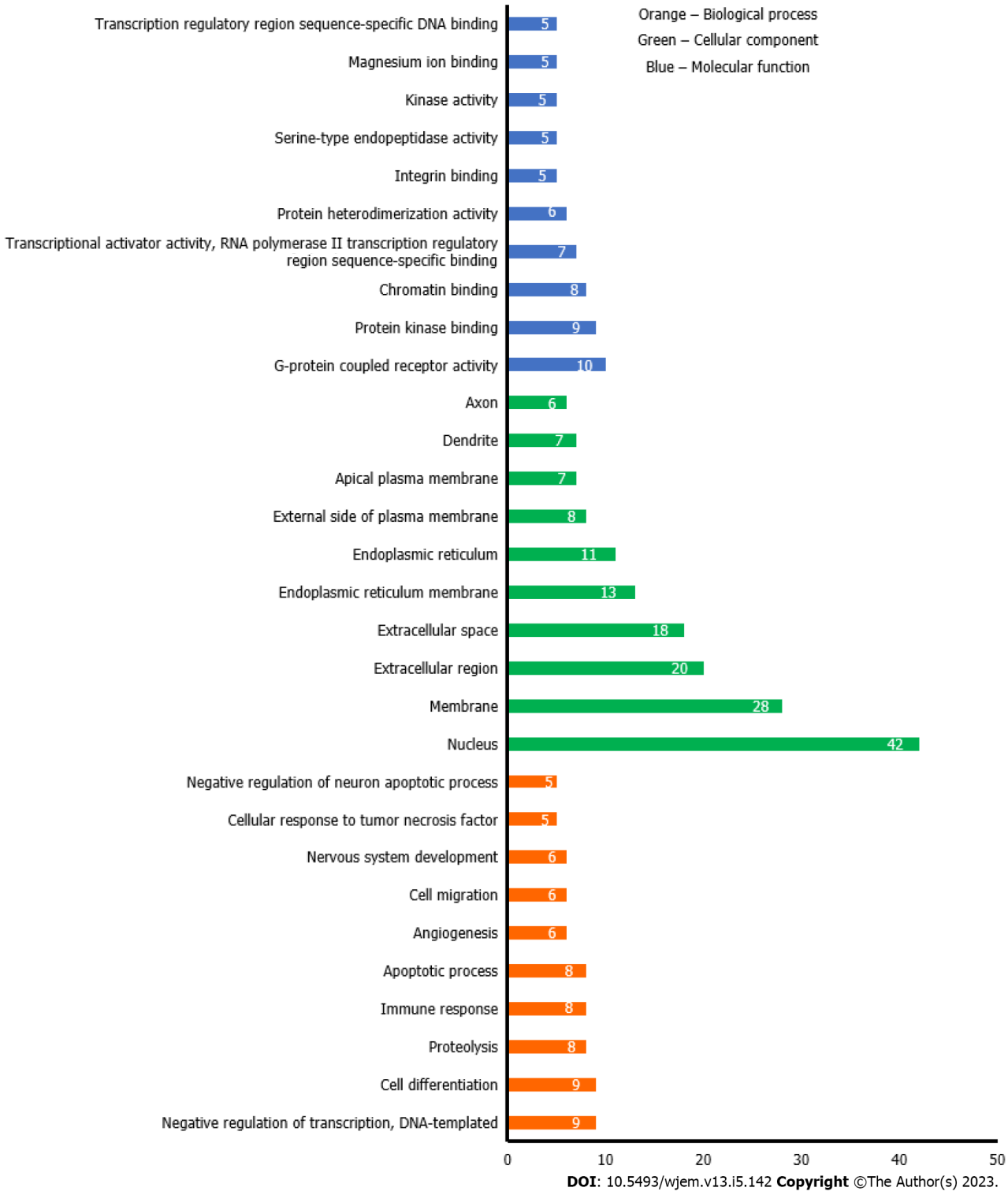


Figure 4 Active ingredient-anti-breast cancer target network of bitter melon.

substituent present on the phenanthrene ring with Met98. As with 1OSF, momordicoside K showed interactions with 6NJS. A hydrogen bond interaction is observed between the phenanthrene ring containing the hydroxyl group and Glu638. Three hydroxyl groups present on the oxane ring form hydrogen bonds with Tyr657, Met660, and Lys658. Also, Van der Waal's interaction and pi-alkyl interaction are observed between the sidechain oxygen atom and methyl substituent, respectively, with Tyr657. Similarly, pi-alkyl interactions between the sidechain and the methyl substituent are present on the sidechain with Ile653 and Tyr640, respectively.

DISCUSSION

In Ayurveda medicine, bitter melon has numerous elements that have therapeutic effects against a variety of ailments. All parts of the plant, including the fruit, are frequently eaten and prepared in many ways, such as stir-frying, stuffing, or using a small amount to add a mildly bitter flavor to soups or beans. Antioxidant, anti-inflammatory, cancer-fighting, anti-diabetic, anti-bacterial, anti-obesity, and immunomodulatory properties are said to be present in the plant. A fascinating discovery showed that bitter melon extract appeared to be beneficial in halting the growth and spread of cancer tumors in studies employing mice models[41]. By causing apoptosis, cell cycle arrest, autophagy, and blocking cancer stem cells, the plant extract reduces the proliferation of cancer cells. The anticancer effects of bitter melon and its

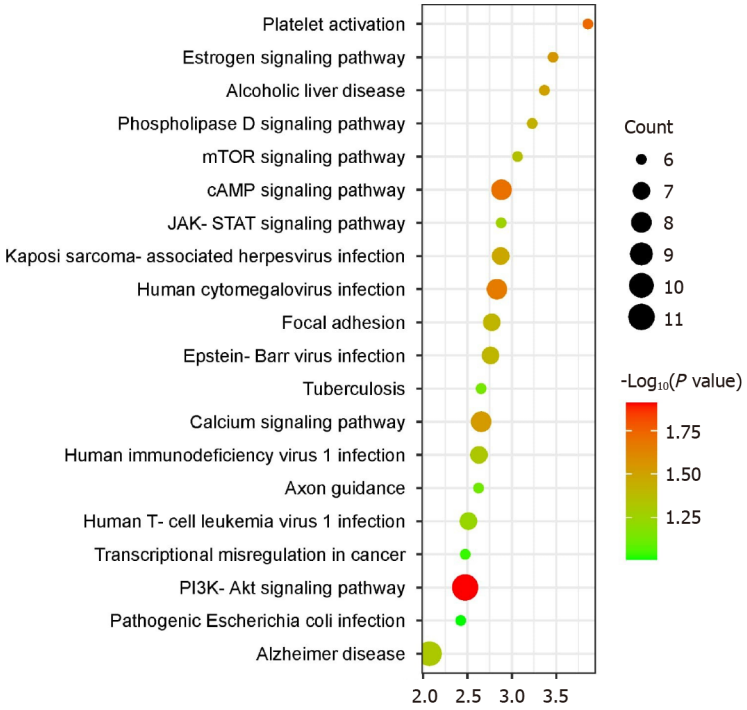


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Figure 5 Gene ontology analysis of anti-breast cancer targets of bitter melon where Y-axis is the number of the targets involved in the gene ontology analysis, and the X-axis is the molecular function, biological process, or cellular component.

active components have been studied; however, the underlying processes have not been comprehensively uncovered. In this study, network pharmacology-an efficient and cost-effective method for conducting systematic therapeutic research-was used to examine the mechanism by which bitter melon treats cancer.

The 133 relevant targets were found by gathering and screening the 14 active components from bitter melon that were screened out from the IMPPAT 2.0 database. The Venn diagram revealed that 112 of the 133 targets connected to bitter melon were also connected to targets for BRCA, indicating that the majority of the pertinent targets in bitter melon were connected to effects that were anti-BRCA. The interactions between different bitter melon targets were visible in the PPI network. The three targets with the top three greatest degree values, HSP90AA1 (43), SRC (42), and STAT3 (38) may be the primary anti-BRCA targets. To further confirm the potential interaction between the important targets and the active compounds, molecular docking was done.



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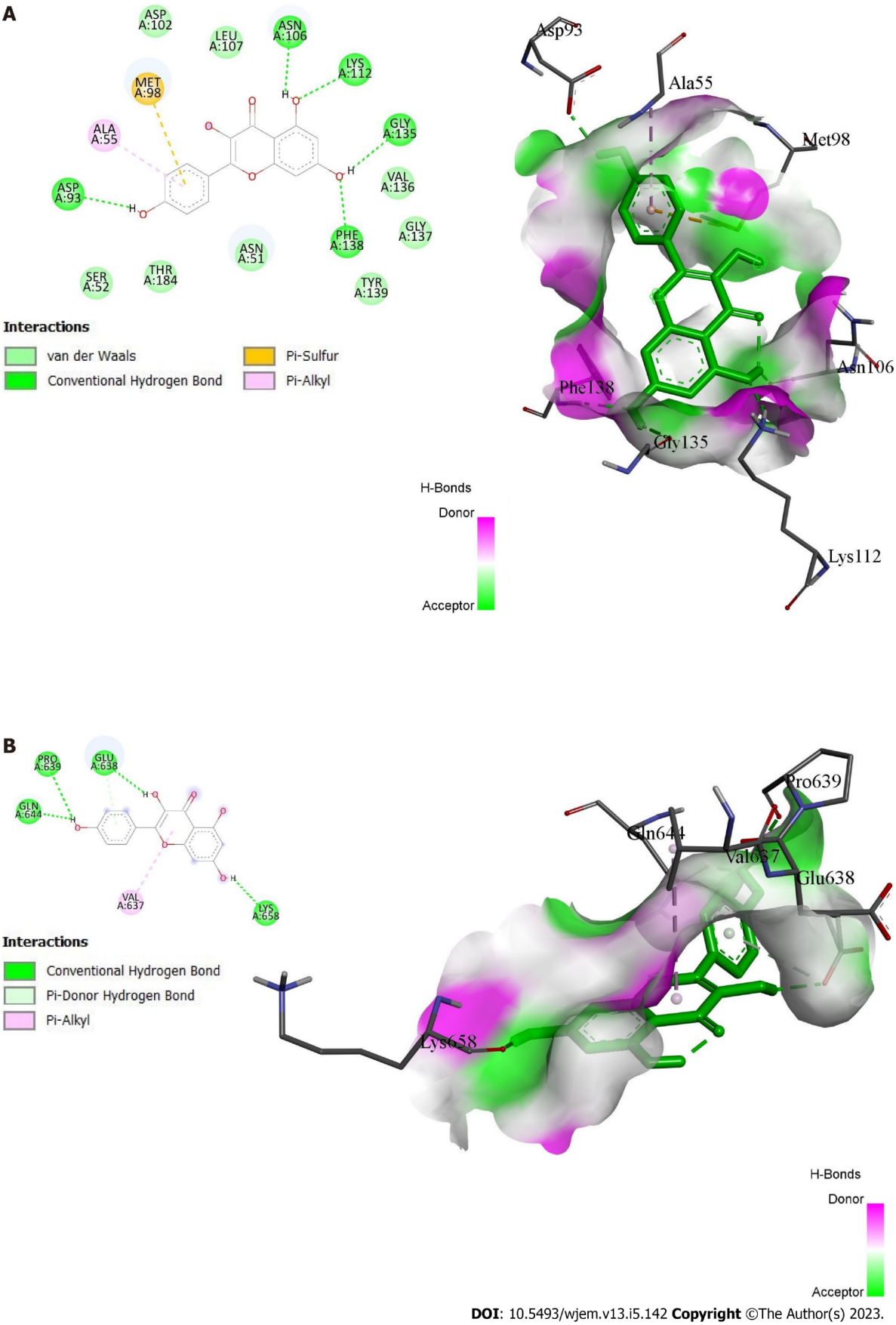
Figure 6 Kyoto encyclopedia of genes and genomes enrichment analysis (top 20) where X-axis is the enrichment gene count, and the Y-axis is the kyoto encyclopedia of genes and genomes pathway. Bubble size represents the number of genes involved in the kyoto encyclopedia of genes and genomes enrichment while color represents -log 10 (*P* value). PIK3-Akt: Phosphatidylinositol 3-kinase/protein kinase B; JAK-STAT: Janus kinase-signal transducer and activator of transcription; mTOR: Mammalian target of rapamycin.

One of the most prevalent proteins in mammalian cells, HSP90, together with the oestrogen receptor, progesterone receptor, key HER2 signaling molecules (HER2, AKT, c-SRC, RAF, and HIF-1), and epidermal growth factor receptor, are crucial for the growth and survival of cancer. Moreover, HSP90 has an N-domain ATP binding site, and all its cellular functions depend on its ATPase activity[11]. HSP90 activation may sustain the malignant phenotype, enhance metastasis, and foster treatment resistance in a variety of BRCA subtypes because it functions as a key integrator of numerous pathways. According to research findings, treating BRCA with up-regulated HSP90 may lessen the chance of fatal recurrence and distant metastases. The transcriptional program that are triggered by various cytokines, growth factors, and carcinogenic stimuli are mediated by the STAT3 protein, which is a signal transducer and transcription activator. Its expression and activity are frequently associated with cellular transformation, tumor development, and tumor initiation. The interleukin 6 (IL-6)/JAK/STAT3 signaling pathway is an actionable target with preclinical and clinical studies demonstrating therapeutic potential in both primary and metastatic BRCA, according to recent findings elucidating the role of IL-6 in BRCA progression, metastasis, and anti-cancer immunity. Importantly, direct targeting of IL-6, IL-6R, gp130 receptor, JAKs, or STAT3 has been examined as a means of blocking the IL-6/JAK/STAT3 signaling axis. Numerous cancers have dysregulated SRC, a non-receptor tyrosine kinase. SRC plays a significant role in a variety of features of tumor formation, including growth, survival, adhesion, migration, invasion, and, most significantly, metastasis in a variety of tumor forms. SRC boosts proliferation, diminishes apoptosis, and promotes metastasis.

The molecular docking results showed that the binding energies of kaempferol to STAT3 and HSP90AA are the highest. Furthermore, momordicoside K and quercetin have good STAT3 and HSP90AA binding properties. The most significant anti-BRCA targets of bitter melon may be STAT3 and HSP90AA. SRC, a member of the family of nonreceptor protein tyrosine kinases, is important for many signaling pathways that are involved in cell growth, survival, motility, and angiogenesis. Compared to STAT3 and HAP90AA, SRC had poor binding activity to kaempferol, momordicoside K, and quercetin, which suggested that SRC may not be the direct target of bitter melon in BRCA treatment.

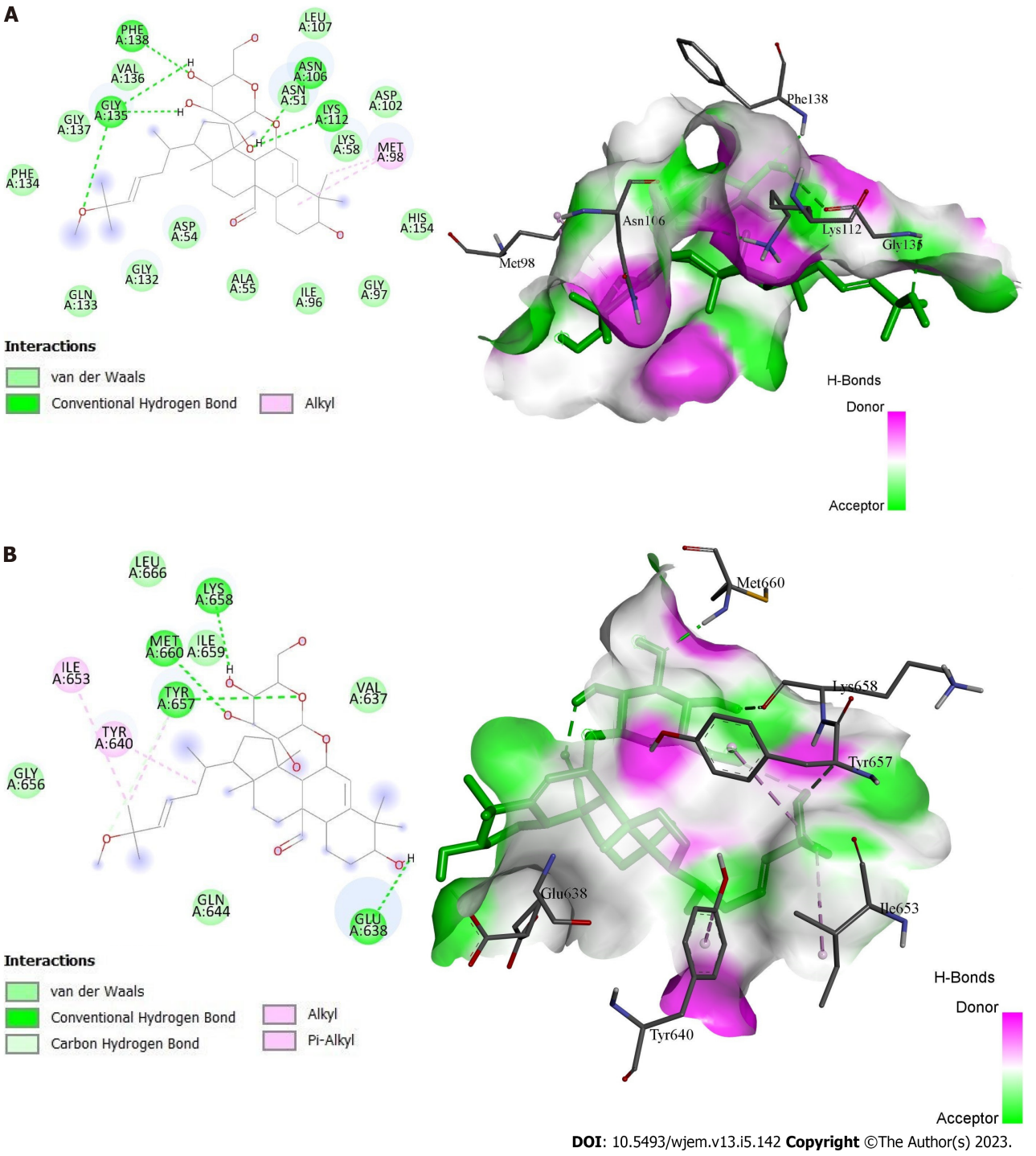
The findings of the KEGG pathway study identified bitter melon's anti-BRCA pathways. Ten out of the top 20 pathways were directly linked to cancer. These pathways, which include PIK3-Akt signaling, transcriptional dysregulation, axon guidance, calcium signaling, focal adhesion, JAK-STAT signaling, cAMP signaling, mTOR signaling, and phospholipase D signaling, may be crucial in the bitter melon's defense against BRCA. According to the findings, bitter melon's targets are crucial in the management of BRCA.

In the PIK3-Akt signaling pathway, which is involved in the control of numerous cellular physiological processes by activating downstream matching effector molecules and is critical for the cell cycle, growth, and proliferation, bitter melon has 11 anti-BRCA targets. Eight anti-BRCA targets in bitter melon are connected to the calcium and cAMP signaling pathways. By the activation of cAMP-dependent protein kinase, the cAMP signaling pathway controls a wide range of intracellular events that are connected to the regulation of cellular proliferation, differentiation, and apoptosis. BRCA development and chemotherapy resistance are both influenced by essential processes regulated by Ca^{2+} signaling pathways, ranging from inflammation to apoptosis. There are seven anti-BRCA targets in the focal adhesion pathway.



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Figure 7 Docking interactions of kaempferol. A: With protein 1OSF; B: With protein 6NJS.



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Figure 8 Docking interactions of momordicoside K. A: With protein 1OSF; B: With protein 6NJS.

bitter melon has six anti-BRCA targets that are involved in the mTOR, JAK-STAT, and phospholipase D signaling pathways. A range of biological processes, including cellular proliferation, survival, metabolism, autophagy, and immunity, are aided by the mTOR signaling pathway. While the phospholipase D signaling system has been linked to survival signaling and the control of cell cycle progression, the JAK-STAT pathway plays critical roles in the carcinogenesis, maintenance, and metastasis of BRCA. As a result, bitter melon has significant therapeutic potential in the treatment of BRCA and functions in several ways.

CONCLUSION

We have successfully investigated the main phytochemicals and molecular processes in bitter melon that are thought to be involved in the therapy of BRCA. This study found 14 major active phytoconstituents as well as 112 anti-BRCA targets in bitter melon. Our research demonstrated that the anti-BRCA benefits of bitter melon are likely caused by negative

regulation of transcription, cell differentiation, apoptosis, proteolysis, negative control of neuron apoptosis, and cell migration. Further, we discovered that nine important pathways, including PIK3-Akt signaling, transcriptional dysregulation, axon guidance, calcium signaling, focal adhesion, JAK-STAT signaling, cAMP signaling, mTOR signaling, and phospholipase D signaling, are likely to be involved in the mechanism of action of bitter melon in the treatment of BRCA. Our results support the hypothesis that bitter melon's potential anti-BRCA properties may be a result of the direct or indirect synergistic effects of multitarget and multi-pathway activities. According to molecular docking research, the principal active phytochemicals in bitter melon may be able to bind to HAP90AA1, STAT3, and other BRCA-related targets. The results of this study offer suggestions for additional investigation into the phytochemicals and mechanisms of bitter melon's anti-BRCA activity. This serves as a foundation for creating cutting-edge anti-BRCA medications based on phytochemicals found in bitter melon but experimental validation is necessary to confirm the predicted therapeutic effects of bitter melon on BRCA. Without experimental evidence, the clinical relevance of these findings remains uncertain.

ARTICLE HIGHLIGHTS

Research background

Bitter melon has been used to stop the growth of breast cancer (BRCA) cells. However, the underlying mechanism is still unclear.

Research motivation

The motivation of this research is to explore the underlying pharmacological mechanisms.

Research objectives

The goal of this study was to predict the therapeutic effect of bitter melon against BRCA using network pharmacology.

Research methods

The active ingredients of bitter melon and the related protein targets were taken from the Indian Medicinal Plants, Phytochemistry and Therapeutics and SuperPred databases, respectively. The GeneCards database has been searched for BRCA-related targets. Through an intersection of the drug's targets and the disease's objectives, prospective bitter melon anti-BRCA targets were discovered. Gene ontology and kyoto encyclopedia of genes and genomes enrichment analyses were carried out to comprehend the biological roles of the target proteins. The binding relationship between bitter melon's active ingredients and the suggested target proteins was verified using molecular docking techniques.

Research results

Through the active ingredient-anti-BRCA target network analysis, three major components were found to be important in mediating the putative anti-BRCA actions of bitter melon: momordicoside K, kaempferol, and quercetin. In the protein-protein interaction network analysis, the top three proteins were determined to be heat shock protein 90 AA, proto-oncogene tyrosine-protein kinase, and signal transducer and activator of transcription 3 (STAT3). According to molecular docking research, the principal active phytochemicals in bitter melon are able to bind to HAP90AA1, STAT3, and other breast cancer-related targets.

Research conclusions

Overall, the integration of network pharmacology, molecular docking, and functional enrichment analyses shed light on potential mechanisms underlying bitter melon's ability to fight BRCA, implicating active ingredients and protein targets, as well as highlighting the major signaling pathways that may be altered by this natural product for therapeutic benefit.

Research perspectives

Database resources were used to examine active compounds found in bitter melon. Network pharmacology and molecular docking techniques were used to explore the mechanism through which bitter melon was used to treat BRCA. Momordicoside K, kaempferol, and quercetin were identified as potentially important active ingredients of bitter melon showing anti-BRCA actions. The study identified several possible molecular targets as well as signaling pathways involved in bitter melon's anti-BRCA actions, like phosphatidylinositol 3-kinase/protein kinase B signaling and janus kinase-signal transducer and activator of transcription signaling. The study suggests further experimental verification to confirm the potential findings of bitter melon in the treatment of BRCA.

FOOTNOTES

Author contributions: Panchal K designed, performed and wrote the paper; Nihalani B designed, performed and wrote the paper; Oza U edited the paper; Panchal A edited the paper; Shah B designed, supervised and edited the paper.

Conflict-of-interest statement: The authors declare no conflicts of interest in this paper.

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Country/Territory of origin: India

ORCID number: Bhumi Shah 0000-0002-7520-7132.

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REFERENCES

1 **Hong R**, Xu B. Breast cancer: an up-to-date review and future perspectives. *Cancer Commun (Lond)* 2022; **42**: 913-936 [PMID: 36074908 DOI: 10.1002/cac2.12358]
2 **Łukasiewicz S**, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast Cancer-Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies-An Updated Review. *Cancers (Basel)* 2021; **13** [PMID: 34503097 DOI: 10.3390/cancers13174287]
3 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]
4 **Anand U**, Dey A, Chandel AKS, Sanyal R, Mishra A, Pandey DK, De Falco V, Upadhyay A, Kandimalla R, Chaudhary A, Dhanjal JK, Dewanjee S, Vallamkondu J, Pérez de la Lastra JM. Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics. *Genes Dis* 2023; **10**: 1367-1401 [PMID: 37397557 DOI: 10.1016/j.gendis.2022.02.007]
5 **Zhang M**, Liu X, Li J, He L, Tripathy D. Chinese medicinal herbs to treat the side-effects of chemotherapy in breast cancer patients. *Cochrane Database Syst Rev* 2007; **2007**: CD004921 [PMID: 17443560 DOI: 10.1002/14651858.CD004921.pub2]
6 **Iqbal J**, Abbasi BA, Mahmood T, Kanwal S, Ali B, Shah SA and Khalil AT. Plant-derived anticancer agents: A green anticancer approach. *Asian Pac J Trop Biomed* 2017; **7**: 1129-1150 [DOI: 10.1016/j.apjtb.2017.10.016]
7 **Liao GS**, Apaya MK, Shyur LF. Herbal medicine and acupuncture for breast cancer palliative care and adjuvant therapy. *Evid Based Complement Alternat Med* 2013; **2013**: 437948 [PMID: 23840256 DOI: 10.1155/2013/437948]
8 **Kung WM**, Lin CC, Kuo CY, Juin YC, Wu PC, Lin MS. Wild Bitter Melon Exerts Anti-Inflammatory Effects by Upregulating Injury-Attenuated CISD2 Expression following Spinal Cord Injury. *Behav Neurol* 2020; **2020**: 1080521 [PMID: 33062068 DOI: 10.1155/2020/1080521]
9 **Prastiyanto ME**, Dewi NMBA, Pratiningtias TD, Pratiwi NMR, Windayani A, Wahyunengsih E, Astuti A, Amir E and Wardoyo FA. In vitro antibacterial activities of crude extracts of nine plants on multidrug resistance bacterial isolates of wound infections. *Biodiversitas* 2021; **22** [DOI: 10.13057/biodiv/d220712]
10 **Takemoto DJ**, Jilka C, Rockenbach S, Hughes JV. Purification and characterization of a cytostatic factor with anti-viral activity from the bitter melon. *Prep Biochem* 1983; **13**: 371-393 [PMID: 6196772 DOI: 10.1080/00327488308068179]
11 **Nerurkar P**, Ray RB. Bitter melon: antagonist to cancer. *Pharm Res* 2010; **27**: 1049-1053 [PMID: 20198408 DOI: 10.1007/s11095-010-0057-2]
12 **Rosyid FN**, Muhtadi M, Hudiyawati D, Sugiyarti S and Rahman AF. Improving Diabetic Foot Ulcer Healing with Adjuvant Bitter Melon Leaf Extract (Momordica charantia L.). *Open Access Maced J Med Sci* 2022; **10**: 122-126 [DOI: 10.3889/oamjms.2022.9503]
13 **Ahmad N**, Hasan N, Ahmad Z, Zishan M and Zohrameena S. Momordica charantia: for traditional uses and pharmacological actions. *J Drug Deliv Ther* 2016; **6**: 40-44 [DOI: 10.22270/jddt.v6i2.1202]
14 **Soo May L**, Sanip Z, Ahmed Shokri A, Abdul Kadir A, Md Lazin MR. The effects of Momordica charantia (bitter melon) supplementation in patients with primary knee osteoarthritis: A single-blinded, randomized controlled trial. *Complement Ther Clin Pract* 2018; **32**: 181-186 [PMID: 30057048 DOI: 10.1016/j.ctcp.2018.06.012]
15 **Saeed F**, Afzaal M, Niaz B, Arshad MU, Tufail T, Hussain MB and Javed A. Bitter melon (Momordica charantia): a natural healthy vegetable. *Int J Food Prop* 2018; **21**: 1270-1290 [DOI: 10.1080/10942912.2018.1446023]
16 **Li Z**, Xia A, Li S, Yang G, Jin W, Zhang M and Wang S. The pharmacological properties and therapeutic use of bitter melon (Momordica charantia L). *Curr Pharmacol Rep* 2020; **6**: 103-109 [DOI: 10.1007/s40495-020-00219-4]
17 **Sasa M**, Inoue I, Shinoda Y, Takahashi S, Seo M, Komoda T, Awata T, Katayama S. Activating effect of momordin, extract of bitter melon (Momordica Charantia L.), on the promoter of human PPARdelta. *J Atheroscler Thromb* 2009; **16**: 888-892 [PMID: 20032574 DOI: 10.5551/jat.2790]
18 **Raina K**, Kumar D, Agarwal R. Promise of bitter melon (Momordica charantia) bioactives in cancer prevention and therapy. *Semin Cancer Biol* 2016; **40-41**: 116-129 [PMID: 27452666 DOI: 10.1016/j.semcancer.2016.07.002]
19 **Ray RB**, Raychoudhuri A, Steele R, Nerurkar P. Bitter melon (Momordica charantia) extract inhibits breast cancer cell proliferation by modulating cell cycle regulatory genes and promotes apoptosis. *Cancer Res* 2010; **70**: 1925-1931 [PMID: 20179194 DOI: 10.1158/0008-5472.CAN-09-3438]
20 **Muhammad N**, Steele R, Isbell TS, Philips N, Ray RB. Bitter melon extract inhibits breast cancer growth in preclinical model by inducing autophagic cell death. *Oncotarget* 2017; **8**: 66226-66236 [PMID: 29029506 DOI: 10.18632/oncotarget.19887]
21 **Shim SH**, Sur S, Steele R, Albert CJ, Huang C, Ford DA, Ray RB. Disrupting cholesterol esterification by bitter melon suppresses triple-negative breast cancer cell growth. *Mol Carcinog* 2018; **57**: 1599-1607 [PMID: 30074275 DOI: 10.1002/mc.22882]

22 **Zhang GB**, Li QY, Chen QL, Su SB. Network pharmacology: a new approach for chinese herbal medicine research. *Evid Based Complement Alternat Med* 2013; **2013**: 621423 [PMID: 23762149 DOI: 10.1155/2013/621423]

23 **Meng Q**, Liu H, Wu H, Shun D, Tang C, Fu X, Fang X, Xu Y, Chen B, Xie Y, Liu Q. A Network Pharmacology Study to Explore the Underlying Mechanism of Safflower (Carthamus tinctorius L.) in the Treatment of Coronary Heart Disease. *Evid Based Complement Alternat Med* 2022; **2022**: 3242015 [PMID: 35607519 DOI: 10.1155/2022/3242015]

24 **Sur S**, Ray RB. Bitter Melon (Momordica Charantia), a Nutraceutical Approach for Cancer Prevention and Therapy. *Cancers (Basel)* 2020; **12** [PMID: 32726914 DOI: 10.3390/cancers12082064]

25 **Mohanraj K**, Karthikeyan BS, Vivek-Ananth RP, Chand RPB, Aparna SR, Mangalapandi P, Samal A. IMPPAT: A curated database of Indian Medicinal Plants, Phytochemistry And Therapeutics. *Sci Rep* 2018; **8**: 4329 [PMID: 29531263 DOI: 10.1038/s41598-018-22631-z]

26 **Kim S**, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker BA, Wang J, Yu B, Zhang J, Bryant SH. PubChem Substance and Compound databases. *Nucleic Acids Res* 2016; **44**: D1202-D1213 [PMID: 26400175 DOI: 10.1093/nar/gkv951]

27 **Waseem D**, Khan GM, Haq IU, Rashid U, Syed DN. The triphenyltin carboxylate derivative triphenylstannyl 2-(benzylcarbamoyl)benzoate impedes prostate cancer progression *via* modulation of Akt/FOXO3a signaling. *Toxicol Appl Pharmacol* 2020; **401**: 115091 [PMID: 32525019 DOI: 10.1016/j.taap.2020.115091]

28 **Gallo K**, Goede A, Preissner R, Gohlke BO. SuperPred 3.0: drug classification and target prediction-a machine learning approach. *Nucleic Acids Res* 2022; **50**: W726-W731 [PMID: 35524552 DOI: 10.1093/nar/gkac297]

29 **Yang Z**, Lu S, Tang H, Qu J, Wang B, Wang Y, Pan G, Rao B. Molecular Targets and Mechanisms of Hedyotis diffusa-Scutellaria barbata Herb Pair for the Treatment of Colorectal Cancer Based on Network Pharmacology and Molecular Docking. *Evid Based Complement Alternat Med* 2022; **2022**: 6186662 [PMID: 35707465 DOI: 10.1155/2022/6186662]

30 **Stelzer G**, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, Stein TI, Nudel R, Lieder I, Mazor Y, Kaplan S, Dahary D, Warshawsky D, Guan-Golan Y, Kohn A, Rappaport N, Safran M, Lancet D. The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. *Curr Protoc Bioinformatics* 2016; **54**: 1.30.1-1.30.33 [PMID: 27322403 DOI: 10.1002/cpbi.5]

31 **Cai H**, Chen H, Yi T, Daimon CM, Boyle JP, Peers C, Maudsley S, Martin B. VennPlex--a novel Venn diagram program for comparing and visualizing datasets with differentially regulated datapoints. *PLoS One* 2013; **8**: e53388 [PMID: 23308210 DOI: 10.1371/journal.pone.0053388]

32 **Szklarczyk D**, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, Doncheva NT, Legeay M, Fang T, Bork P, Jensen LJ, von Mering C. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res* 2021; **49**: D605-D612 [PMID: 33237311 DOI: 10.1093/nar/gkaa1074]

33 **Wang CR**, Chen HW, Li Y, Zhou MY, Wong VK, Jiang ZH, Zhang W. Network Pharmacology Exploration Reveals Anti-Apoptosis as a Common Therapeutic Mechanism for Non-Alcoholic Fatty Liver Disease Treated with Blueberry Leaf Polyphenols. *Nutrients* 2021; **13** [PMID: 34836315 DOI: 10.3390/nu13114060]

34 **Liu YY**, Yu LH, Zhang J, Xie DJ, Zhang XX, Yu JM. Network Pharmacology-Based and Molecular Docking-Based Analysis of Suanzaoren Decoction for the Treatment of Parkinson's Disease with Sleep Disorder. *Biomed Res Int* 2021; **2021**: 1752570 [PMID: 34660782 DOI: 10.1155/2021/1752570]

35 **Qian X**, Zhang L, Xie F, Cheng Y, Cui D. Network-Based Pharmacological Study on the Mechanism of Guishao-Liujun Decoction in the Treatment of Gastric Cancer. *Front Pharmacol* 2022; **13**: 937439 [PMID: 35865953 DOI: 10.3389/fphar.2022.937439]

36 **Zhou G**, Feng X, Tao A. Explore the Lipid-Lowering and Weight-Reducing Mechanism of Lotus Leaf Based on Network Pharmacology and Molecular Docking. *Evid Based Complement Alternat Med* 2021; **2021**: 1464027 [PMID: 34745273 DOI: 10.1155/2021/1464027]

37 **Habib I**, Anjum F, Mohammad T, Sulaimani MN, Shafie A, Almehmadi M, Yadav DK, Sohal SS, Hassan MI. Differential gene expression and network analysis in head and neck squamous cell carcinoma. *Mol Cell Biochem* 2022; **477**: 1361-1370 [PMID: 35142951 DOI: 10.1007/s11010-022-04379-3]

38 **Dennis G Jr**, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol* 2003; **4**: P3 [PMID: 12734009]

39 **Khan SA**, Lee TKW. Network-Pharmacology-Based Study on Active Phytochemicals and Molecular Mechanism of Cnidium monnieri in Treating Hepatocellular Carcinoma. *Int J Mol Sci* 2022; **23** [PMID: 35628212 DOI: 10.3390/ijms23105400]

40 **Sriramulu DK**, Wu S and Lee SG. Effect of ligand torsion number on the AutoDock mediated prediction of protein-ligand binding affinity. *J Ind Eng Chem* 2020; **83**: 359-365 [DOI: 10.1016/j.jiec.2019.12.009]

41 **Fang EF**, Zhang CZ, Wong JH, Shen JY, Li CH, Ng TB. The MAP30 protein from bitter gourd (Momordica charantia) seeds promotes apoptosis in liver cancer cells in vitro and in vivo. *Cancer Lett* 2012; **324**: 66-74 [PMID: 22579806 DOI: 10.1016/j.canlet.2012.05.005]

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LETTER TO THE EDITOR

Research on nanosciences involvement in pharmaceutical education should be reinforced

Zheng-Wei Huang, Ye-Qi Huang

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Zheng-Wei Huang, Ye-Qi Huang, College of Pharmacy, Jinan University, Guangzhou 510006, Guangdong Province, China

Corresponding author: Zheng-Wei Huang, PhD, Assistant Professor, Associate Professor, College of Pharmacy, Jinan University, No. 855 East Xingye Dadao, Panyu District, Guangzhou 510006, Guangdong Province, China. huangzhengw@jnu.edu.cn

Abstract

Inclusion of nanoscience in pharmaceutical education should be reinforced, in order to match the demand of current pharmaceutical talent cultivation.

Key Words: Nanosciences; Pharmaceutical education; Talent cultivation; Reformation

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Core Tip: Nanosciences have currently boosted the development of various disciplines, including pharmaceutical sciences. Theoretically, nanosciences should be involved in pharmaceutical education, in order to cultivate pharmaceutical talents familiar with nanosciences. However, the current courses on nanoscience are insufficient in pharmaceutical curricula, and only a few studies on this topic are documented in databases. It seems that this field is rarely exploited. We therefore urge investigators and educators to perform more studies, and include nanosciences in pharmaceutical education.

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TO THE EDITOR

Since the 1990s, nanoscience has gained rapid momentum in research and development. Although nanotoxicology is a rising concern worldwide[1], and the ethical issues (so-called "nanoethics")[2] and the ecological risks[3] of nanotechnology have been identified, the application of nanomaterials and nanotechnologies is still a focus.

Consequently, various nanomaterials and nanotechnologies have been developed and introduced into different fields, including the chemical, biological, environmental, medical, and pharmaceutical sciences. In the chemistry field, it is possible to apply nanotechnologies to various chemical compounds, including polymers, to modify their structure and function. In a recent study, metal-organic framework based nano-adsorbents have made a number of noteworthy advances in anti-chemical warfare reagents[4]. In the biology field, one of the most acclaimed achievements in nanotechnology in molecular biology is identification of the vaccination mechanism for coronavirus disease 2019 (COVID-19) using nanoscale vector systems[5]. In the environment field, nanotechnology is utilized not only to enhance the environment but also to produce renewable sources of energy. A paradigmatic example is the employment of nanofluids in solar cells which can produce electricity at a competitive cost[6]. In the medical field, the successful application of nanomedicine has helped to develop enhanced versions of diagnostics, treatment, prevention, and proactive healthcare measures[7]. Recent research has shown that the controllability of nanorobots has advanced, allowing for efficient remodeling of dense tumor stromal microenvironments to enable deep tumor penetration[8].

As pharmaceutical researchers, the authors of this paper consider that nanoscience is deeply involved in the field of pharmaceutical sciences. The following are several key examples: (1) Pharmaceutical chemistry: One-component new chemical entity nanomedicines are synthesized to enhance therapeutic efficacy[9]; (2) Pharmacology: Nanovesicles can be used as analytical tools to investigate cellular signaling pathways[10]; (3) Pharmaceutics: Nanoparticles are effective carriers for drug loading and delivery[11]; and (4) Pharmaceutical analysis: Nanotechnologies can facilitate the separation, identification, and quantification of drug molecules[12]. This information is schematically illustrated in Figure 1.

As nanoscience has become an ever more integral part in pharmaceutical sciences, individuals with pharmaceutical interests (*i.e.*, pharmaceutical scientists, pharmacists, staff in the pharmaceutical industry, and governors in drug administration) should master the relevant knowledge and skills regarding nanoscience. Pharmaceutical education is the fundamental and vital approach for cultivating pharmaceutical talent; hence, pharmaceutical curricula should involve imparting knowledge and skills on nanoscience. However, for several pharmaceutical education systems worldwide, the core courses in pharmaceutical curricula remain focused on the subdisciplines of pharmaceutical sciences (such as pharmaceutical chemistry, pharmacology, pharmaceutics, and pharmaceutical analysis[13] and fundamental chemistry (such as inorganic, organic, and physical chemistry and biochemistry[14], and courses focused on nanoscience are insufficient or even absent. The reasons for this may include the following: Nanotechnology is a cutting-edge research field; its novelty may present some challenges to the faculty. Even in a nanoscience or nanotechnology training program, some teachers show a preference for teaching more familiar courses so that they can apply the knowledge into the classroom in a timely manner[15]. It is documented that low self-confidence, associated with a lack of knowledge on the new content, sometimes hinders the acceptance and the willingness to use it in the classroom[16]. Another aspect is that the study of basic theoretical subjects is still generally considered necessary in pharmacy education. Some people therefore reject the educational importance of nanoscience, believing that there is no room for a new science curriculum like that[17].

Moreover, not only are current courses lacking in coverage of nanoscience, retrospective/progressive/prospective studies on the inclusion of nanoscience in pharmaceutical education are also in their infancy. The authors consulted six databases associated with Web of Science (Web of Science Core Collection, Chinese Science Citation Database, KCI-Korean Journal Database, Medline, ProQuest Dissertations & Theses Citation Index, and SciELO Citation Index) using various search sets (Table 1) on July 13, 2023. Documents published between 1965 and 2023 were searched, and all document types and languages were included. Duplicate and irrelevant publications were manually excluded. However, according to the results of the literature survey, only eight documents were retrieved, which was surprisingly low. For comparison, the number of documents on pharmaceutical education and nanoscience were 50537 and 2985487, respectively. These results are shown in a Venn diagram in Figure 2. From the authors' perspective, characterized by interdisciplinarity, nanoscience is a deeply interconnected discipline, encompassing diverse areas of modern science and technology. When taking nanoscience into the classroom, it has been confronted with the dilemma of whether it should be taught as a new or a subsidiary discipline. Accordingly, the intricate nature of nanoscience may be the possible reason that has resulted in a scarcity of research exploring its intersection with a specialized field such as pharmacy education.

Overall, the current scenario is that insufficient attention has been paid to the inclusion of nanoscience into pharmaceutical education. Consequently, the cultivation of pharmaceutical talents mastering the required knowledge and skills of nanoscience cannot be guaranteed. With the continuous development of nanoscience, its inclusion in pharmaceutical science will become more comprehensive in the future. It is worth noting that the market for nanomedicines for disease management has great potential. The global market for nanomedicine was estimated at $53 billion in 2009. It is expected to grow by 13.5% to reach $100 billion in recent years[18]. These data demonstrate global interest in the nanoscience field. Therapeutic formulations utilizing nanotechnology hold potential for improving clinical outcome. Engineered nanomaterials are rapidly evolving in drug development, and offer promise in overcoming biological barriers and achieving precise drug delivery for precision medicine[19]. In addition, the potential of nanotechnology in pharmacy will be further expanded with efforts to combine nanomaterials with some established formulations. Recent studies have pointed out that the combined properties of hydrogels and nanoparticles in smart nanogels can improve drug loading capacity, drug stability, target delivery and therapeutic efficacy[20]. Considering these factors, it is foreseeable that nanotechnology will become more widely and tightly integrated into pharmaceuticals in the future. Thus, correspondingly, continued efforts are required to promote the inclusion of nanoscience in pharmaceutical education.

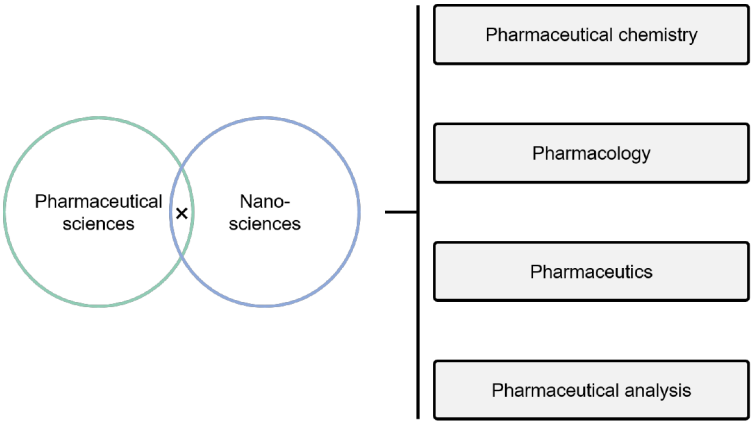
To achieve this aim, the authors propose three suggestions:

Firstly, colleges and universities should establish scientific foundations for the educational reformation of nanoscience courses; the role of foundations in support of the education process is essential. A positive example to be studied is the National Science Foundation (NSF). The NSF is charged with funding basic research programs to maximize the

Table 1 Search sets for literature retrieval

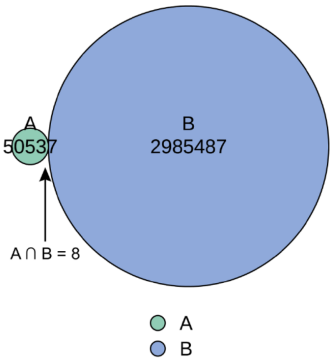
Step	Search set	Interpretation
#1	TS = pharmacy educat[1], TS = pharmaceutical educat[1], TS = pharmacy train[1], TS = pharmaceutical train[1], TS = pharmacy curricul[1], TS = pharmaceutical curricul[1]	Pharmaceutical education as the topic
#2	TS = nano[1]	Nano-related terms as the topic
#3	#1 AND #2	Intersection of #1 and #2

[1]Is an infinite truncator, which allows the term to be infinitely expanded.



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Figure 1 Main applications of nanoscience and nanotechnology in pharmaceutical sciences.



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Figure 2 Euler Venn diagram showing the intersection of the publications on pharmaceutical education (A) and nanoscience (B).

advancement of science in the United States through the development of scientific information. In the National Nanotechnology Initiative, NSF assumed responsibility for funding basic research and education in nanoscience and nanotechnology, leading to a healthy growth of nanotechnology in the United States[21]. Referring to the operation and supporting model of NSF, sufficient attention from colleges and universities should be given to the investment of adequate funding to meet the development of nanoscience in pharmacy education.

Secondly, faculties must initiate changes to their curricular systems and add nanoscience courses. In the post-pandemic era, the rate of change in healthcare has rapidly accelerated. Consequently, healthcare professionals must dedicate themselves to lifelong learning through continuing education and professional development programs, including those associated with nanoscience[22]. For instance, teachers should learn about the applications of nanotechnology in COVID-19 treatment, and pass this knowledge to the students in the classroom. Through these efforts, students can gain scientific and technological literacy, which has a significant impact on curriculum design[15]. It can also serve as an effective means of bridging the gap between workforce needs and cutting-edge fields[23].

Lastly, journals, such as the *World Journal of Experimental Medicine*, should encourage the submission of relevant studies as a publishing platform. Currently, the evaluation of papers by impact factor is still the dominant approach used by journals. However, this single-factor approach has led to much discussion about its update or revolution[24], and the

actual implication of papers for the real world should be reconsidered. As a publishing platform, considering and encouraging papers based on multiple factors may be a positive guide for the conduct of research focusing on a rare field (the very scenario of nanoscience education in pharmacy). The authors envision that the degree of inclusion of nanoscience in pharmaceutical education can be increased in the near future.

In summary, nanoscience is rapidly evolving in a number of disciplines and fields. It has been widely used in the fields of chemistry, biology, environment, medicine, and pharmacy, and has attracted much attention. Especially in the field of pharmacy, nanoscience and nanotechnology have played a significant role. However, at present, the coverage of nanoscience in pharmaceutical courses and educational studies is lacking, which is detrimental to the cultivation of talents in this field. The gaps in this area should be further addressed by all groups. We propose three suggestions to boost the inclusion of nanoscience in pharmacy education: (1) Colleges and universities should establish scientific foundations for the educational reformation of nanoscience courses; (2) faculties must initiate changes to their curricular systems and add nanoscience courses; and (3) journals should encourage the submission of relevant studies as a publishing platform. Similar to the situation in pharmacy education, clinical medicine training should also include more courses on nanotechnology, and we will be conducting in-depth research on this topic in the future.

FOOTNOTES

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ORCID number: Zheng-Wei Huang 0000-0003-2351-7347.

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REFERENCES

1 **Jamil B**, Javed R, Qazi AS, Syed MA. Nanomaterials: Toxicity, Risk Managment and Public Perception. In: Rai M, Biswas JK, editors. Nanomaterials: Ecotoxicity, Safety, and Public Perception. *Cham: Springer International Publishing* 2018; 283-304 [DOI: 10.1007/978-3-030-05144-0_14]

2 **Mohanta D**, Ahmaruzzaman M. Addressing Nanotoxicity: Green Nanotechnology for a Sustainable Future. In: Hussain CM, editor. The ELSI Handbook of Nanotechnology: Risk, Safety, ELSI and Commercialization. *Scrivener Publishing LLC* 2020; 103-116 [DOI: 10.1002/9781119592990.ch6]

3 **Zafar H**, Javed R, Zia M. Nanotoxicity assessment in plants: an updated overview. *Environ Sci Pollut Res Int* 2023; **30**: 93323-93344 [PMID: 37544947 DOI: 10.1007/s11356-023-29150-z]

4 **Song Y**, Peng C, Iqbal Z, Sirkar KK, Peterson GW, Mahle JJ, Buchanan JH. Graphene Oxide and Metal-Organic Framework-Based Breathable Barrier Membranes for Toxic Vapors. *ACS Appl Mater Interfaces* 2022; **14**: 31321-31331 [PMID: 35771504 DOI: 10.1021/acsami.2c07989]

5 **Kisby T**, Yilmazer A, Kostarelos K. Reasons for success and lessons learnt from nanoscale vaccines against COVID-19. *Nat Nanotechnol* 2021; **16**: 843-850 [PMID: 34381200 DOI: 10.1038/s41565-021-00946-9]

6 **Hussein AKJR**, Reviews SE. Applications of nanotechnology to improve the performance of solar collectors – Recent advances and overview. *Renew Sustain Energy Rev* 2016; **62**: 767-792 [DOI: 10.1016/j.rser.2016.04.050]

7 Sandow J. Drug Discovery and Evaluation: Methods in Clinical Pharmacology: Drug Discovery and Evaluation: Methods in Clinical Pharmacology; 2011

8 **Yan M**, Chen Q, Liu T, Li X, Pei P, Zhou L, Zhou S, Zhang R, Liang K, Dong J, Wei X, Wang J, Terasaki O, Chen P, Gu Z, Jiang L, Kong B. Site-selective superassembly of biomimetic nanorobots enabling deep penetration into tumor with stiff stroma. *Nat Commun* 2023; **14**: 4628 [PMID: 37532754 DOI: 10.1038/s41467-023-40300-2]

9 **Ma Z**, Li J, Lin K, Ramachandran M, Zhang D, Showalter M, De Souza C, Lindstrom A, Solano LN, Jia B, Urayama S, Duan Y, Fiehn O, Lin TY, Li M, Li Y. Author Correction: Pharmacophore hybridisation and nanoscale assembly to discover self-delivering lysosomotropic new-chemical entities for cancer therapy. *Nat Commun* 2021; **12**: 2013 [PMID: 33767181 DOI: 10.1038/s41467-021-22419-2]

10 **Kadry MO**, Abdel Megeed RM. Ubiquitous toxicity of Mercuric Chloride in target tissues and organs: Impact of Ubidecarenone and liposomal-Ubidecarenone STAT 5A/PTEN/PI3K/AKT signaling pathways. *J Trace Elem Med Biol* 2022; **74**: 127058 [PMID: 35952450 DOI: 10.1016/j.jtemb.2022.127058]

11 **Nel J**, Elkhoury K, Velot É, Bianchi A, Acherar S, Francius G, Tamayol A, Grandemange S, Arab-Tehrany E. Functionalized liposomes for

targeted breast cancer drug delivery. *Bioact Mater* 2023; **24**: 401-437 [PMID: 36632508 DOI: 10.1016/j.bioactmat.2022.12.027]

12 **Wang YF**, Pan MM, Yu X, Xu L. The Recent Advances of Fluorescent Sensors Based on Molecularly Imprinted Fluorescent Nanoparticles for Pharmaceutical Analysis. *Curr Med Sci* 2020; **40**: 407-421 [PMID: 32681246 DOI: 10.1007/s11596-020-2195-z]

13 **Zheng H**, Hu BJ, Sun Q, Cao J, Liu FM. Applying a Chemical Structure Teaching Method in the Pharmaceutical Analysis Curriculum to Improve Student Engagement and Learning. *J Chem Educ* 2020; **97**: 421-426 [DOI: 10.1021/acs.jchemed.9b00551]

14 **Ali RM**, Idoudi S, Abdulrahman N, Mraiche F. Biotechnology content in pharmacy curricula: Focus on Arab Countries. *Curr Pharm Teach Learn* 2023; **15**: 654-660 [PMID: 37481456 DOI: 10.1016/j.cptl.2023.07.001]

15 **Spyrtou A**, Manou L, Peikos G. Educational Significance of Nanoscience–Nanotechnology: Primary School Teachers' and Students' Voices after a Training Program. *Educ Sci (Basel)* 2021; **11**: 724 [DOI: 10.3390/educsci11110724]

16 **Bitan-Friedlander N**, Dreyfus A, Milgrom Z. Types of "teachers in training": the reactions of primary school science teachers when confronted with the task of implementing an innovation. *Teach Teach Educ* 2004; **20**: 607-619 [DOI: 10.1016/j.tate.2004.06.007]

17 **Healy N**. Why Nano Education? *J Nano Educ* 2009; **1**: 6-7 [DOI: 10.1166/jne.2009.004]

18 **Mittal D**, Ali A, Md S, Baboota S, Sahni JK, Ali J. Insights into direct nose to brain delivery: current status and future perspective. *Drug Deliv* 2014; **21**: 75-86 [PMID: 24102636 DOI: 10.3109/10717544.2013.838713]

19 **Mitchell MJ**, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. *Nat Rev Drug Discov* 2021; **20**: 101-124 [PMID: 33277608 DOI: 10.1038/s41573-020-0090-8]

20 **Bhaladhare S**, Bhattacharjee S. Chemical, physical, and biological stimuli-responsive nanogels for biomedical applications (mechanisms, concepts, and advancements): A review. *Int J Biol Macromol* 2023; **226**: 535-553 [PMID: 36521697 DOI: 10.1016/j.ijbiomac.2022.12.076]

21 **Lukishova S**, Bigelow N. Undergraduate program in nanoscience and nanoengineering: five years after the National Science Foundation grant including two pandemic years. *Optical Engineering* 2022; **61**: 081810 [DOI: 10.1117/1.oe.61.8.081810]

22 **Lok P**, Beyene K, Awaisu A, Woods D, Kheir N. Microcredentials training in pharmacy practice and education: an exploratory study of its viability and pharmacists' professional needs. *BMC Med Educ* 2022; **22**: 332 [PMID: 35488266 DOI: 10.1186/s12909-022-03341-7]

23 **Hart D**. Closing the Nanotechnology Workforce Gap [Nano Safety and Education]. *IEEE Nanotechnology Magazine* 2012; **6**: 27-28 [DOI: 10.1109/MNANO.2012.2192656]

24 **Rousseau R**. Updating the journal impact factor or total overhaul? *Scientometrics* 2012; **92**: 413-417 [DOI: 10.1007/s11192-012-0649-1]



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