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MINIREVIEWS

Joint replacement and human immunodeficiency virus

Maryam Salimi, Peyman Mirghaderi, Seyedarad Mosalamiaghili, Ali Mohammadi, Amirhossein Salimi

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Abstract

The incidence of human immunodeficiency virus (HIV)-infected cases that need total joint replacement (TJR) is generally rising. On the other hand, modern management of HIV-infected cases has enabled them to achieve longevity while increasing the need for arthroplasty procedures due to the augmented degenerative joint disease and fragility fractures, and the risk of osteonecrosis. Although initial investigations on joint replacement in HIV-infected cases showed a high risk of complications, the recent ones reported acceptable outcomes. It is a matter of debate whether HIV-infected cases are at advanced risk for adverse TJR consequences; however, the weak immune profile has been associated with an increased probability of complications. Likewise, surgeons and physicians should be aware of the complication rate after TJR in HIV-infected cases and include an honest discussion of the probable unwelcoming complication with their patients contemplating TJR. Therefore, a fundamental review and understanding of the interaction of HIV and arthroplasty are critical.

Key Words: Human immunodeficiency virus; Arthroplasty; Infection; Joint replacement

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Core Tip: The outcome and prevalence of complications are controversial among human immunodeficiency virus-infected cases who need arthroplasty. According to our literature review, total joint replacement procedures are recommended based on patient-specific factors such as viral load, $CD4^+$ T-cell count, clinical classification, history of intravenous drug addiction, and the patient's overall health. Optimization with antiviral drugs is also suggested before elective arthroplasty.

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INTRODUCTION

Human immunodeficiency virus (HIV) infection in humans was initially recognized in the early 1980s and documented as the reason for acquired immunodeficiency syndrome (AIDS). With an estimated 37 million affected people in 2015, the pandemic has grown to all corners of the world. Southern and Eastern Africa have tolerated the extreme impact of this problem, with 52% of the total infected people, 19 million cases, and more than 1 million new annual infections. The yearly occurrence of newly infected cases has decreased by 38%, from 2001 (3.4 million) to 2015 (2.1 million), with the successful advent and introduction of antiretroviral therapy (ART) programs in the last years[1,2]. AIDS-related expiries have also dropped from a high in 2005 (2.0 million), distinguished to be the topmost of the epidemic, to 1.1 million yearly in 2015, which means a reduction of about 45%[3].

The etiology varies according to highly active antiretroviral treatment (HAART) and disease-related mechanisms[4].

Femoral head osteonecrosis was initially reported in HIV-infected cases in the early 1990s - before the ART time. Several investigations have considered ART drugs as an independent factor for osteonecrosis development[5-7]. Therefore, HIV-positive individuals would need TJR at a much earlier age due to the osteonecrosis of the hip, in comparison to their counterparts who are affected by osteoarthritis and mostly are bilaterally involved[8].

Furthermore, a reduction in the density of bone minerals is more frequently seen in HIV-positive cases compared to non-infected controls[9,10]. The stimulation of interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) is followed by an imbalance of the receptor stimulator of nuclear factor kappa-B (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) system, which eventually motivates bone resorption by osteoclasts and inhibits osteoclastic action. The cascade mentioned above in the long term accelerates osteopenia and would augment the probability of fragility fractures, particularly fractures of the femur neck in HIV-infected males[11].

It is controversial whether HIV-infected cases are at advanced menace for adverse TJR consequences; however, the weak immune profile may have been correlated to the increased probability of complications. Although initial investigations on hip and knee arthroplasty in HIV-infected cases showed a high risk of complications, the recent ones reported acceptable outcomes[12]. Despite the increasing number of the HIV-infected population, there is scarce literature investigating outcomes after TJR in infected ones. Periprosthetic joint infection (PJI) is one of the most prevalent causes of revision arthroplasties, and its underlying risk factors could be both non-modifiable and modifiable. Non-modifiable factors include chronic diseases such as cirrhosis, coagulopathies, kidney disease, and obstructive pulmonary as well as race, gender, and age[13,14]. Modifiable issues have been proven to increase early revision rates and complications and prolong the hospital stay. These factors include diabetes, smoking, opioid use, poor dentition, obesity, HIV, and *Staphylococcus aureus* colonization[14]. A bunch of risk factors may also have been developed in HIV-positive cases undergoing TJR[15]. Initial studies in orthopedic trauma patients infected with HIV showed a significant rate of postoperative infection rate[16,17]. Parvizi *et al* [18] demonstrated a similar finding in arthroplasty cases. However, their study involved patients with hemophilia, which may have been demonstrated as an independent risk factor for PJI[19,20]. Further investigations among non-hemophilic HIV-positive cases have displayed a remarkably lower rate of infection but a worryingly high rate of total complications[1].

Furthermore, Orthopedic Infections International Consensus in 2018 announced that HIV is an independent factor in increasing the risk of PJI[21]. However, the importance was reduced when HAART managed HIV-positive cases, and preoperative optimization was performed for them[21]. Therefore, identification and optimization of HIV-positive patients before surgery are mandatory to reduce the burden of already serious healthcare systems.

According to the above, a fundamental approach and understanding of the TJR and HIV interaction are inevitable. Therefore, we conduct a literature review on joint replacement in the HIV-positive group. Moreover, this minireview highlights the critical aspect of complications and outcomes.

CLASSIFICATION

Clinical staging methods help determine the prognosis and treatment for HIV-positive patients. Orthopedic surgeons who plan to operate on these patients must understand these systems well. The two most commonly used HIV classification systems were introduced by the Centers for Disease Control (CDC) and the World Health Organization (WHO). In 2014, the CDC released the modified definition of surveillance case for HIV infection[22]. This surveillance case explanation for HIV has been revised and combined into a sole case definition for people of every age. New multi-test algorithms, such as HIV-I and HIV-II detection criteria and direct detection of HIV infection, have been added to laboratory criteria to define an approved case. The surveillance case definition refers to monitoring the burden of HIV infection at the population level and planning for care and prevention, not for individual patient treatment decisions. A proven case of HIV infection is classified into five stages (0, 1, 2, 3, or unknown) (Table 1).

The CDC proposes three descriptive classifications based on the presence or absence of specific clinical symptoms (Table 2). The $CD4^+$ T-cell count further subdivides these three types. $CD4^+$ T-cell counts above 500/μL are categorized as types A1, B1, and C1. $CD4^+$ T-cell counts within 200/μL and 400/μL are A2, B2, and C2 categories. Infections in patients having $CD4^+$ T-cell numbers lower than 200/μL are classified as A3, B3, or C3.

Orthopedic surgeons mostly use the WHO staging system[23]. Patients are divided into four clinical groups using this classification system: Stage one patients are asymptomatic or accompanied by persistent generalized lymphadenopathy; patients with stage two present with a slight disease along with 10% weight loss and skin exhibition; those with stage three experienced moderate disease and weight loss of more than 10%, severe bacterial infections, and chronic diarrhea lasting more than a month; and patients with the last stage have a severe disease, AIDS, cryptosporidiosis, toxoplasmosis, HIV wasting syndrome, Kaposi's sarcoma, and pneumonia caused by *Pneumocystis jirovecii*.

PATHOPHYSIOLOGY AND ASSOCIATION BETWEEN HIV AND TJR

In HIV-infected persons, osteonecrosis is still the most common reason for TJR. Since the first case was reported in 1990, the annual osteonecrosis prevalence among HIV-infected ones has consistently grown, ranging from 0.08% to 1.33%[24,25]. Although the specific origin of osteonecrosis in HIV-positive people is unknown, various risk variables have been identified[26,27].

Alcohol, corticosteroids, hyperlipidemia, anticardiolipin and antiphospholipid antibodies, cigarette smoking, course of HIV infection, and antiretroviral therapy are some of the most common risk factors for changes in the bloodstream to the bone[26,27]. Although many researchers believe that the multiple effects of several factors that impair bone blood flow would lead to osteonecrosis, some investigators provide evidence that HIV infection alone can cause osteonecrosis[7,22,28]. According to a study by Ries *et al*[28], there was a much higher percentage of HIV-infected individuals with nontraumatic osteonecrosis without any identified risk factors than HIV-negative cases. Figure 1 demonstrates factors affecting joint replacement among HIV-infected patients.

Some factors stimulate osteonecrosis in both HIV-positive and -negative cases. Steroids alter bone metabolism and the growth of bone marrow stromal cells, resulting in fat penetration into the bone marrow and blockage of the bloodstream inside the bone. The cascade above increases intraosseous pressure. Moreover, steroids may cause acute fat embolization in capillary arteries[23-26], which are widely used to manage HIV-related diseases, such as pneumocystis pneumonia and also central nervous system toxoplasmosis[29-31]. Miller *et al*[29] also verified the abovementioned association and determined that even short-term consumption of a corticosteroid drug could significantly raise the incidence of osteonecrosis. Hyperlipidemia, a known subsequent event of HIV infection and HAART diets, is substantially implicated in atherosclerotic pathways and is known to be a causative agent in steroid-induced osteonecrosis. Several investigations have linked high serum cholesterol levels and the consumption of fat-reduction drugs to the pathogenesis of osteonecrosis[7,26,31].

HIV has a detrimental effect on the density of the bone mineral, and patients with HIV infection are prone to develop osteoporosis 3.7 times greater than non-infected people[1,32]. Consequently, there is an augmented fragility fracture risk in HIV-positive cases[1]. Low bone mineral density is associated with low $CD4^+$ T-cell count and long-term HIV infection[1]. Another reason for generated osteopenia is the constant inhibition of osteoblasts and activation of osteoclasts, which is a result of miss-balanced receptor activation of the nuclear factor kappa-B (RANK)/RANKL/OPG system[11,33]. This pattern is triggered by the virus, which causes activation of TNF-α and IL-1[10].

Increased levels of antiphospholipid antibodies and further thrombophilic factors are correlated with osteonecrosis in HIV-infected cases. Antiphospholipid antibodies are considered a risk factor for venous and arterial thrombosis and are generally found in HIV-infected patients. In a new investigation, the incidence of antiphospholipid antibodies in HIV-infected cases was predicted to be around 44%[34]. It is well known that these antibodies are involved in the progression of osteonecrosis in systemic lupus erythematosus patients, and similar processes have been suggested for HIV-infected cases[25,35].

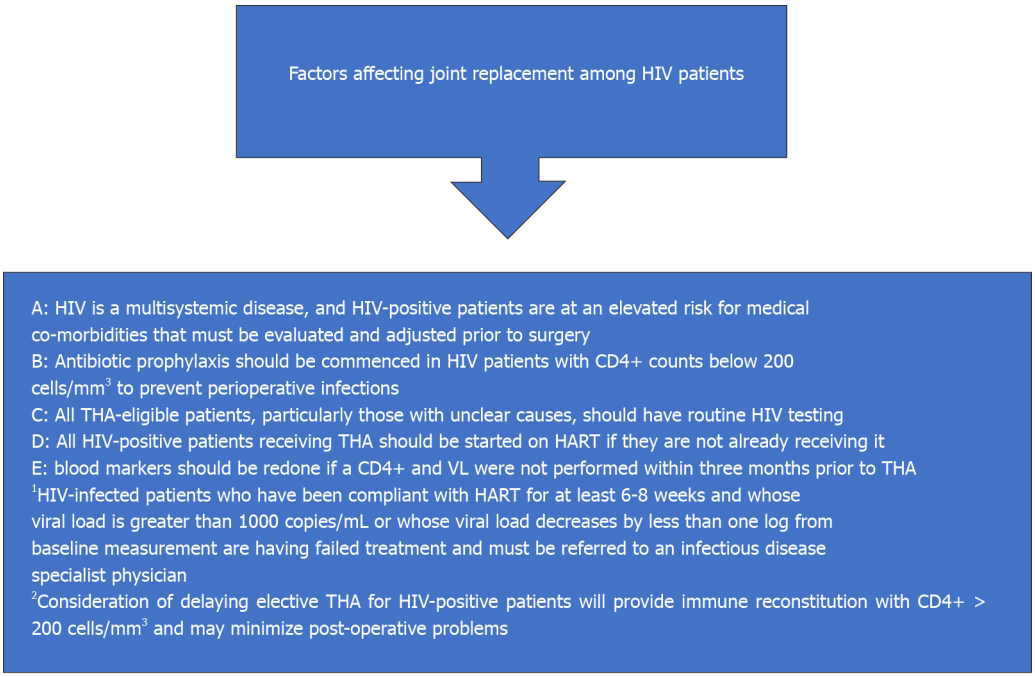
Table 1 Classification of laboratory categories based on age-specific CD4+ T-lymphocyte count

Age on date of CD4+ T-lymphocyte test						
Stage	< 1 yr		1–5 yr		≥ 6 yr	
	Cells/μL	%	Cells/μL	%	Cells/μL	%
1	≥ 1500	≥ 34	≥ 1000	≥ 30	≥ 500	≥ 26
2	750-1499	26-33	500-999	22-29	200-499	14-25
3	< 750	< 26	< 500	< 22	< 200	< 14

Table 2 Classification clinical category

Clinicalcategory	Symptomatic conditions
A	Asymptomatic HIV infection, persistent generalized lymphadenopathy, acute (primary) HIV infection complicated by illness, or a history of acute HIV infection. Bacillary angiomatosis
B	Candida infection, cervical dysplasia, constitutional symptoms, hairy leukoplakia, herpes zoster, unexplained thrombocytopenic purpura, listeriosis, pelvic inflammatory disease, and peripheral neuropathy.
C	AIDS-defining diseases: Toxoplasmosis, Kaposi's sarcoma, Pneumocystis pneumonia

HIV: Human immunodeficiency virus; AIDS: Acquired immunodeficiency syndrome.



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Figure 1 Factors affecting joint replacement among human immunodeficiency virus-positive patients. HIV: Human immunodeficiency virus; THA: Total hip arthroplasty.

Recent studies have engrossed the role of antiretroviral therapies, mainly protease inhibitors, in the pathogenesis of osteonecrosis. Even though the precise processes of HAART that may play a role in osteonecrosis are unclear, the association of protease inhibitors and conventional osteonecrosis risk factors has been recognized[8,22,26]. Hypertriglyceridemia due to HAART has been widely cited as a potential cause of osteonecrosis. Through interactions with cytochrome P450, protease inhibitors may increase the effectiveness of steroid therapy. A study by Penzak *et al*[36] showed that treatment with ritonavir dramatically amplified the concentration of prednisolone in healthy cases. Several HIV-positive cases with osteonecrosis were reported before the introduction of HAART, suggesting that HIV treatment regimens could not be the primary cause of the disease. Osteonecrosis has a complex etiology and is highly prevalent among HIV-positive individuals, thus increasing the need for total joint arthro-

plasties in this patient population.

While the incidence of HIV infection is steadily increasing, broad access to HAART has upgraded from 25% in 2010 to 73% in 2021[37]. Increased access to HAART has reduced global AIDS-related mortality by 64% since its peak in 2004 and by 47% since 2010[37]. HIV and HAART are independently associated with joint pathology that ultimately requires joint replacement[4].

HAART should be started in all HIV-positive patients, regardless of clinical stage or $CD4^+$ T-cell count [1]. This is particularly significant for cases pending elective TJR. First-line HAART almost always consists of a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) and an agent of a different drug class. Protease inhibitors (PIs) are believed to form the primary drug class that aids avascular necrosis (AVN) in the hip[4]. In addition, drugs containing tenofovir have been linked to the progression of osteopenia[1]. Remarkably, all first-line regimens in every corner of the world include tenofovir agents.

Due to osteodegenerative and renal complication of tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF) was developed[38]. TAF continues to cause renal disease and bone loss, but to a lesser extent than TDF. The WHO recommended that integrase strand transfer inhibitors (InSTIs) replace PIs as the ideal first-line regimen. However, more than half of the global HIV prevalence is in East and South Africa, which cannot easily access InSTIs[38].

There are several side effects to HAART drugs, and the patient's diet should be monitored. There are significant drug interactions between anesthetic drugs and NRTIs and PIs that are usually prescribed for induction and sedation. TDF is nephrotoxic, so patients need to be checked for blood urea nitrogen; the urine protein-to-creatine ratio is sometimes assessed to evaluate their renal function[39]. Anemia and neutropenia are side effects of zidovudine. Thus, these patients need a complete blood count, white cell count, and differential amount[40].

EVALUATION AND PREOPERATIVE OPTIMIZATION

Different studies show how immune system impairment is a key factor in postoperative risk of infection and complications[41]. Any major disease which causes impaired immune system like diabetes mellitus, which results in impaired leukocyte function[42,43], and even malnutrition[44], can be risk factors for PJI[41].

$CD4^+$ T-cell count is an alternative indicator of immunological status, and the number of $CD4^+$ T cells less than 200 cells/mm^3 confirms the diagnosis of AIDS[39]. Optimistic monitoring of the patient's immune status is essential in managing HIV-infected patients undergoing HAART. The opportunistic infections-related prevalence, mortality, and morbidity are higher in patients with a CD4+ T-cell count of 200 cells/mm^3[45]. Patients with opportunistic infections and abandoned viral status should be recognized to reduce perioperative and postoperative complications[45]. Antibiotic prophylaxis should be initiated for all patients with a $CD4^+$ T-cell count < 200 cells/mm^3[45]. The guidelines recommend trimethoprim-sulfamethoxazole (TMP-SMX) as an effective treatment for opportunistic infections[45].

Viral load (VL) is a reliable indicator of treatment effectiveness and is influenced by patient adherence to HAART. Elective procedures should be delayed for those with higher VL[45]. If, after 6 to 8 wk of careful treatment, the VL is greater than 1000 copies/mL or less than one log below starting point, there is a viral failure[45].

Multiple systems can be affected by HIV, and patients should be thoroughly evaluated for perioperative risk classification before TJR. HIV-positive cases often suffer cardiovascular disease, nephropathy, liver disaster, neurological problems, and non-AIDS-related malignancies[46]. Evaluation of the patients mentioned before surgery should contain suitable blood work, nutritional status, and immune deficiency syndrome stage[47]. In HIV-positive patients, postoperative complications are mainly due to immunodeficiency, not the procedure itself. Therefore, a comprehensive evaluation is required to prevent anesthetic and surgery problems[45].

HIV-infected cases who developed advanced stages of the disease should also have their nutritional status evaluated since there is a higher risk for nutritional deficiencies and wasting[45]. Hypoalbuminemia is an independent risk factor for mortality in HIV-infected cases postoperatively compared to the non-infected control group[48].

Malnutrition is related to several problems following THA, such as delayed wound healing and prolonged wound damage, which increases the risk of infection[21]. If the diet is inadequate, dietary supplements may be necessary and should be consulted with a nutritionist[45].

On the other hand, compared to the HIV-negative control group, HIV-positive individuals are at higher risk for coronary artery disease, which may be due to persistent chronic inflammation[45]. HAART drugs have decreased the risk of perioperative cardiovascular problems[46].

HIV-infected patients treated with HAART have an increased incidence of insulin resistance, diabetes, and hypercholesterolemia. This may increase the risk of AVN in the femoral head and thus upsurge the need for TJR among HIV-infected cases. All HIV-infected patients should undergo a thorough clinical evaluation for lipodystrophy and fasting lipograms, mainly if they consume a HAART diet containing PI[45].

In HIV-positive patients, smoking is approximately 40% more prevalent than in the general population, and the probability of quitting is also lower[49]. This makes the patients susceptible to atherosclerotic developments, chronic lung disease, and postoperative respiratory infections[45]. Before elective surgery, the respiratory function should be evaluated in all HIV-infected smokers to determine their capacity for diffusion[45]. In addition, AIDS patients are susceptible to nosocomial invasive bacterial infections, and elective TJR should be delayed to optimize immune system reconstruction[45].

OUTCOMES OF TJR IN HIV-INFECTED PATIENTS

As the global number of HIV-infected cases rises, the need to evaluate the results of orthopedic treatment in them also increases. Due to the growing demand for these treatments, the consequences and complications of TJR among them are also receiving particular attention. In addition to functional implications, many of these studies have investigated the incidence of postoperative infections. Previously, HIV was known as an independent factor for infection after surgery. Therefore, the benefits and safety of elective surgery in these patients have been questioned. However, some recent investigations of TJR in HIV-infected patients have raised doubts about this theory.

The treatment algorithms for HIV-positive patients should be similar to algorithms used for non-infected cases, with two-step revision surgery. We suggest a long course of intravenous antibiotics: Up to 6 mo *vs* 6 wk. The medical condition of patients, especially their VL and CD4+ T-cell count, should always be considered.

Several studies focusing on the results of complete joint arthroplasty in HIV-infected cases have focused on hemophilic ones. Due to recurrent periarticular and intra-articular hemorrhage, people with hemophilia are at a much higher risk for joint degeneration. The risk of developing joint arthropathy increased with the higher rates of HIV infection in these people between 1979 and 1985 due to the application of polluted blood products. During that period, injection of infected factor VIII resulted in serum conversion in about 80% of hemophilia patients. Preliminary research conducted by Gregg-Smith *et al*[50] and Wiedel *et al*[51], evaluated the infection prevalence in HIV-infected hemophiliac cases who underwent complete knee arthroplasty before the extensive use of HAART. Wiedel *et al*[51], in a 1989 study, found an advanced rise in acute postoperative infections. The result, as mentioned earlier, was also confirmed by Gregg-Smith *et al*[50].

Hicks *et al*[52] and several other researchers confirmed that the growing risks are related to TJR in HIV-positive hemophiliacs, linking the probability of these problems to the number of $CD4^+$ T-cells. Hicks *et al*[52] evaluated the results of 102 TJR in 73 hemophiliac patients infected by HIV in a large multicenter retrospective study. The incidence of surgical site infection was 18.7% for initial surgeries and 36.3% for revision after an average five-year follow-up. About 62.5% of the infected group had a preoperative $CD4^+$ T-cell count of less than 0.2×10^9/L, while 16.7% were non-infectious. Ragni *et al*[19] found a similar increase in the rate of postoperative infections in HIV-positive hemophiliac patients with CD4+ T-cell counts of less than 0.2×10^9/L in patients who underwent knee and hip arthroplasty surgeries. An infection rate of 15% was observed in a retrospective analysis of 115 hemophilia clinics in the United States in 1995, which the authors highlighted was three times more likely than the risk of infection at surgical sites among HIV-negative arthroplasty patients.

In contrast, many current investigations could not confirm the extensive risk of problems in this population of patients after total joint replacement. For instance, Powell *et al*[53] evaluated the incidence of postoperative infections following total TJR of the knee and hip in HIV-negative and HIV-positive hemophilia patients between 1975 and 2002. Three of the 30 joints among HIV-infected cases developed primary joint infections, compared to two of the 21 non-infected groups. The perusal above did not show an elevated relative risk of surgical site infection in HIV-positive patients (relative risk = 1.49). The researchers concluded that TJR is a viable treatment choice for hemophilic patients with concomitant HIV infection.

The relative safety of the orthopedic procedures in HIV-positive hemophiliac patients was further supported by Unger *et al*[54], who evaluated 26 knee arthroplasties of 15 patients with HIV infection and type A hemophilia; all of them experienced an improvement in function after arthroplasty, and also during 6.4 years of follow-up, no infection occurred at the surgical site.

Subsequent research has evaluated the outcomes of TJR procedures in HIV-infected adults who are not hemophiliacs. For instance, Parvizi *et al*[18] conducted a study on total hip and knee arthroplasties in 21 infected cases and reported a significant incidence of postoperative complications. Twelve of 21 arthroplasties needed to be reconsidered at the subsequent evaluation due to recurrent infection. Furthermore, there was a significant relationship between the immunological status of patients and the probability of deep infection (six joints). The authors mentioned above discovered a significant prevalence of *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* among deep-seated infections[18].

In parallel to prior investigations, Habermann *et al*[55] have compared the incidence of total complications in non-hemophiliac infected cases and hemophiliac patients after TJR. However, they found no significant difference in functional outcomes[55].

Recently, Mahoney *et al*[56] conducted a study among non-hemophiliac HIV-infected patients who had total hip arthroplasty, demonstrating favorable functional results. Three years afterward, only one in 40 patients with a history of intravenous drug abuse had severe infection with antibiotic-resistant *Staphylococcus aureus*.

Moreover, further comorbidities associated with HIV, including intravenous drug abuse, negatively and significantly influence the clinical outcome of TJR surgery. On the other hand, Lehman *et al*[57] assessed the risk of PJI in HIV-infected patients and simultaneous infection from IV drug use (IVDU) following TJR. In the study mentioned above, patients with co-infection of HIV and IVDU had a more than 40% surgical site infection rate. Infections did not occur in four HIV-positive patients without comorbidities (IVDU, hemophilia). The findings of this study suggest an individual-based evaluation of the benefits and risks of TJR.

THA AND HAART IN HIV-INFECTED PATIENTS UNDERGOING TJR

The functional consequences of THA in HIV-positive individuals are controversial in the literature. There is still considerable debate about the relative efficacy and safety of TJR in these patients. It was traditionally believed that HIV infection was associated with an augmented complication risk, particularly infectious adverse effects. In addition, the growing use of HAART has reduced the related complications. HIV-infected patients who do not receive HAART are more prone to unfavorable outcomes. Parvizi *et al*[18] found a substantial complication rate among HIV-infected cases who had undergone total hip arthroplasty; it is worth mentioning that 80% of them did not receive HAART. This amount included 29% of PJIs[58]. The mean $CD4^+$ T-cell count for those who developed PJI was 239 cells/mm^3, while the mean for the entire study population was 523 cells/mm^3[58]. A similar result was displayed by Lehman *et al*[57]; they reported a PJI rate of 14.3% among HIV-infected cases receiving THA. It is worth mentioning that none of them were optimized by HAART.

Several studies have shown ambiguous functional results when comparing HIV-positive patients treated with HAART with the HIV-negative control group. Graham *et al*[59] conducted a survey among 43 THAs which had been performed on 29 HIV-positive patients in 2014; they reported no occurrences of dislocations, deep or superficial infections, or venous thrombotic events, either in late (> 6 wk) or early (< 6 wk) follow-ups. Significant postoperative functional benefits and tangible progress in the Harris hip scores were recorded. Between 1998 and 2010, likewise, Naziri *et al*[60] conducted a study among 9275 HIV-positive patients with THA to evaluate the outcomes of THA and compare it to the results of 2.7 million non-infected cases. The rates of minor and major complications in HIV-infected individuals were 5.2% and 2.9%, respectively, meaningfully higher than the respective rates of 4.8% and 2.7% in non-infected individuals. In addition, a longer duration of hospitalization was observed in the HIV-positive group[60].

Sadoghi *et al*[61] analyzed global registration data from New Zealand, Australia, Denmark, Finland, Norway, and Sweden, showing that aseptic loosening is the main reason for revising THAs, which accounts for 55.7% of them. PJI was the third cause of revision[61]. The onset of aseptic loosening does not appear to be due to HIV[1,46].

One- and five-year survival rates after THA surgery in HIV-positive patients are similar to those of non-HIV-positive patients[1]. Issa *et al*[62] evaluated the lifespan of prostheses after ten years; there was no difference in the survival of infected and non-infected cases. Moreover, Novikov *et al*[63] showed that a significant amount of revision THA (80%) happened during the first year after surgery. Still, the revision rate in the long-term analysis was similar to that among non-infected patients[1]. Although functional outcomes of infected cases optimized with HAART are equal to those of HIV-negative cases, a higher probability of PJI has been reported.

Previously, THA outcomes in HIV-positive individuals with simultaneous hemophilia[19] or IVDU [57] showed a high proportion of poor outcomes and late deep infections. In a comprehensive assessment of 722 THAs out of 25 research studies, Enayatollahi *et al*[58] found that PJI is significantly more prevalent among those with simultaneous HIV infection and hemophilia than in HIV-infected alone. The corresponding PJI rates were 10.98% and 2.28%, respectively. Nevertheless, before HAART, the frequency of PJI was up to 50%[58]. The onset of HAART coincided with a reduction in the incidence of THA infections in HIV-positive individuals[46,58]. Also, Youngman *et al*[64] found that the complication rate of HIV-positive patients who were not treated with the HAART and did not undergo THAs due to femoral head osteonecrosis is 12.5% higher than that of patients who were optimized with HAART.

ELECTIVE SURGERY

Given the increasing need for TJR and the HIV pandemic, it is essential to determine if a threshold should be proposed for elective arthroplasty. King *et al*[48] evaluated 30-d postoperative THA mortality in the United States retrospectively and found that HIV-positive patients had a higher mortality rate

(3.4%) than HIV-negative patients (1.6%). Regardless of $CD4^+$ T-cell count, the HIV-positive group had a higher mortality rate than the control group, even though lower $CD4^+$ T-cell count was correlated with a greater mortality rate[48]. There is a hypothesis that a $CD4^+$ T-cell count threshold of 200 cells/mm^3 is associated with a higher risk of postoperative complications, such as PJI. However, this has not been proven[1,46].

There is considerable evidence that HIV-positive cases, particularly the poorly controlled ones, are more susceptible to postoperative complications[46]. Serum markers should be monitored periodically, as the infection is perilous, mainly if $CD4^+$ T-cell counts decrease[46,65]. Many studies have not linked the $CD4^+$ T-cell count consistently and accurately. Dimitriou *et al*[1] reported that THA might be safely administered to HIV-positive patients regardless of their $CD4^+$ T-cell status, while Shah *et al*[46] suggested a $CD4^+$ T-cell count of more than 400 cells/mm^3 was essential for a safe THA. On the other hand, Sax *et al*[66] suggested that elective THA can be done in both groups of HIV-infected patients who are actively taking ART or not, and the complication rates are similar to those not infected by HIV.

Preoperative VL may be more important than $CD4^+$ T-cell count. A higher level of the virus may indicate unsuccessful surgery and require a referral to an infectious disease specialist. Horberg *et al*[47] conducted a retrospective study on more than 5000 HIV-infected individuals; they believed that VL > 30000 copies/mL was related to a 3-fold bigger chance of postoperative complications.

In a systematic evaluation, Shah *et al*[46] recommended a VL of 50 copies/mL before elective procedures. It is suggested that patients continue the HAART regimen, and full compliance is essential. If the subsequent studies show a decrease in $CD4^+$ T-cell count or an increase in VL, treatment mismatch or failure should be considered.

CONCLUSION

Currently, reports on HIV-positive patients and TJR afford contradictory information. While some authors reported significant complications and reconsideration rates following TJR on HIV-positive patients, most of these collections include a large proportion of HIV-infected cases with hemophilia. Thus, it is a critical confounder in the results described in prior studies. Future studies should evaluate the results of HIV-positive patients with no simultaneous disease to identify its risks, especially for patients with complete joint arthroplasty. Before surgery, we now examine each patient and try to optimize the general health of HIV-positive patients. Total joint replacement procedures are recommended based on patient-specific factors such as viral load, $CD4^+$ T-cell count, clinical classification, history of intravenous drug addiction, and the patient's overall health. The risks and frequency of perioperative problems should be made clear to patients. We expect TJR to improve the quality of life of these patients. In Figure 1, we have recommendations for better outcomes of TJR in HIV-positive cases.

FOOTNOTES

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Abstract

The liver has many significant functions, such as detoxification, the urea cycle, gluconeogenesis, and protein synthesis. Systemic diseases, hypoxia, infections, drugs, and toxins can easily affect the liver, which is extremely sensitive to injury. Systemic infection of severe acute respiratory syndrome coronavirus 2 can cause liver damage. The primary regulator of intracellular pH in the liver is the Na^+/H^+ exchanger (NHE). Physiologically, NHE protects hepatocytes from apoptosis by making the intracellular pH alkaline. Severe acute respiratory syndrome coronavirus 2 increases local angiotensin II levels by binding to angiotensin-converting enzyme 2. In severe cases of coronavirus disease 2019, high angiotensin II levels may cause NHE overstimulation and lipid accumulation in the liver. NHE overstimulation can lead to hepatocyte death. NHE overstimulation may trigger a cytokine storm by increasing proinflammatory cytokines in the liver. Since the release of proinflammatory cytokines such as interleukin-6 increases with NHE activation, the virus may indirectly cause an increase in fibrinogen and D-dimer levels. NHE overstimulation may cause thrombotic events and systemic damage by increasing fibrinogen levels and cytokine release. Also, NHE overstimulation causes an increase in the urea cycle while inhibiting vitamin D synthesis and gluconeogenesis in the liver. Increasing NHE3 activity leads to Na^+ loading, which impairs the containment and fluidity of bile acid. NHE overstimulation can change the gut microbiota composition by disrupting the structure and fluidity of bile acid, thus triggering systemic damage. Unlike other tissues, tumor necrosis factor-alpha and angiotensin II decrease NHE3 activity in the intestine. Thus, increased luminal Na^+ leads to diarrhea and cytokine release. Severe acute respiratory syndrome coronavirus 2-induced local and systemic damage can be improved by preventing virus-induced NHE overstimulation in the liver.

Key Words: Liver; Hepatocyte; Severe acute respiratory syndrome coronavirus 2; COVID-19; Na^+/H^+ exchanger; Sodium-proton pump

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Core Tip: Severe acute respiratory syndrome coronavirus 2 readily infects the liver by angiotensin-converting enzyme 2. Increased angiotensin II causes Na^+/H^+ exchanger (NHE) overstimulation allowing the accumulation of Na^+ and Ca^{2+} in hepatocytes. Thus, hepatocytes are damaged and eventually die. Increased cytokine release increases fibrinogen levels, enhancing thrombotic events. Cytokine storms can be triggered by NHE overstimulation. Severe acute respiratory syndrome coronavirus 2-induced NHE overstimulation can change bile acid structure, which disrupts gut microbiota and can trigger cytokine storms. Liver damage from the virus can be considered the most important cause of disease progression and mortality.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a worldwide pandemic and multisystem organ involvement, resulting in immense hospital costs and mortality. Although the virus settles in the lungs and causes infection, it spreads to other organs expressing angiotensin-converting enzyme 2 (ACE2), such as the heart, liver, and kidney, *via* the neighborhood route, systemic blood circulation, or vascular endothelium[1,2]. Liver involvement is also often seen in the course of severe novel coronavirus disease 2019 (COVID-19) and may advance the progression of the disease[3]. Understanding how SARS-CoV-2 spreads to these organs and solving the damage mechanism in the organs will provide an outstanding opportunity to reduce the severity and mortality of the disease.

Intracellular pH plays a vital role in SARS-CoV-2 infection[4]. Physiologically, the pH of endosomes is low. Therefore, acidic pH leads to the autophagy of viruses and harmful substances[4]. However, SARS-COV-2 can smoothly escape autophagy by manipulating cellular autophagy[5] and cause infection at low intracellular pH by fusing with ACE2[6]. When the pH of the endosomes becomes alkaline, SARS-CoV-2 cannot infect the cell since the configuration of ACE2 changes[7]. Hydroxychloroquine, which makes the intracellular pH alkaline, has been used until its harmful effects appear in patients with COVID-19[8]. The primary regulator of intracellular pH in the liver and many organs is the Na^+/H^+ exchanger (NHE)[9,10]. In a previous study, NHE activity was high in the blood of patients with COVID-19[11]. NHE activation in COVID-19 has been associated with cytokine storms and organ damage[12]. Unraveling the unique relationship between NHE and SARS-CoV-2 may illuminate liver involvement, mortality, and progression of COVID-19. Therefore, we should solve the mechanism of possible SARS-CoV-2 and NHE interaction in liver tissue.

COVID-19 AND THE LIVER

The liver has many significant functions, such as detoxification, the urea cycle, gluconeogenesis, and protein synthesis. Many systemic diseases, hypoxia, infections, drugs, and toxins can easily affect the liver, which is extremely sensitive to injury. Systemic infection of SARS-CoV-2 or agents used in COVID-19 treatment can cause liver damage[13]. Liver enzyme elevation is seen in 16.1%-53.1% of patients with COVID-19[14]. Liver damage has been seen in approximately 20% of hospitalized patients with COVID-19[14]. Since hepatocytes express ACE2, SARS-CoV-2 can directly cause liver damage[15].

In COVID-19, gamma-glutamyl transferase (GGT) and alkaline phosphatase, which indicate cholestasis, increase. Aspartate aminotransferase (AST) and alanine transaminase (ALT), which are markers of hepatocyte damage, increase as well[16]. AST, ALT, GGT, and bilirubin indicate liver damage but also reflect the severity of COVID-19[13,17]. Liu *et al*[16] revealed that GGT and ACE2 share the same transcriptional machinery and speculated that GGT may indicate the *in vivo* expression level of ACE2. In addition, the finding of high bilirubin as a predictive marker for mortality in patients with COVID-19 reveals the importance of liver involvement in the disease[18,19]. On the other hand, the presence of microthrombus in most patients with COVID-19 and the fact that arterial thrombosis is

responsible for mortality indicate that liver involvement is more significant in COVID-19.

Fibrinogen, the precursor of fibrin, is synthesized in the liver. D-dimer, a fibrin degradation product, was elevated in many critically ill COVID-19 patients[20]. D-dimer levels correlate with total bilirubin, AST, and ALT levels in patients with COVID-19[21]. Baroiu *et al*[21] reported that D-dimer might be a predictive marker of abnormal liver function parameters and liver injury in patients with COVID-19. Therefore, in most patients with COVID-19, liver involvement may be responsible for mortality and the disease progression.

NHE AND ITS ISOFORMS

There are many ion pumps in plasma membranes. They provide signaling and stabilization of ion concentrations between intracellular and extracellular areas. NHE is one of the most important and has nine isoforms. These isoforms are localized in different tissues and cell types and have various functions depending on their localization. NHE1 is the cleaning form found in almost all tissues and is expressed abundantly in the liver. NHE2 is located in the stomach and intestines. Liver, intestine, and kidney tissues have NHE3. NHE4 is in the stomach and kidney. The brain has NHE5. NHE6-9 are found in intracellular organelles[22-24]. NHEs are involved in the etiology of several gastrointestinal and liver diseases[22].

NHE AND ITS FUNCTION IN THE LIVER

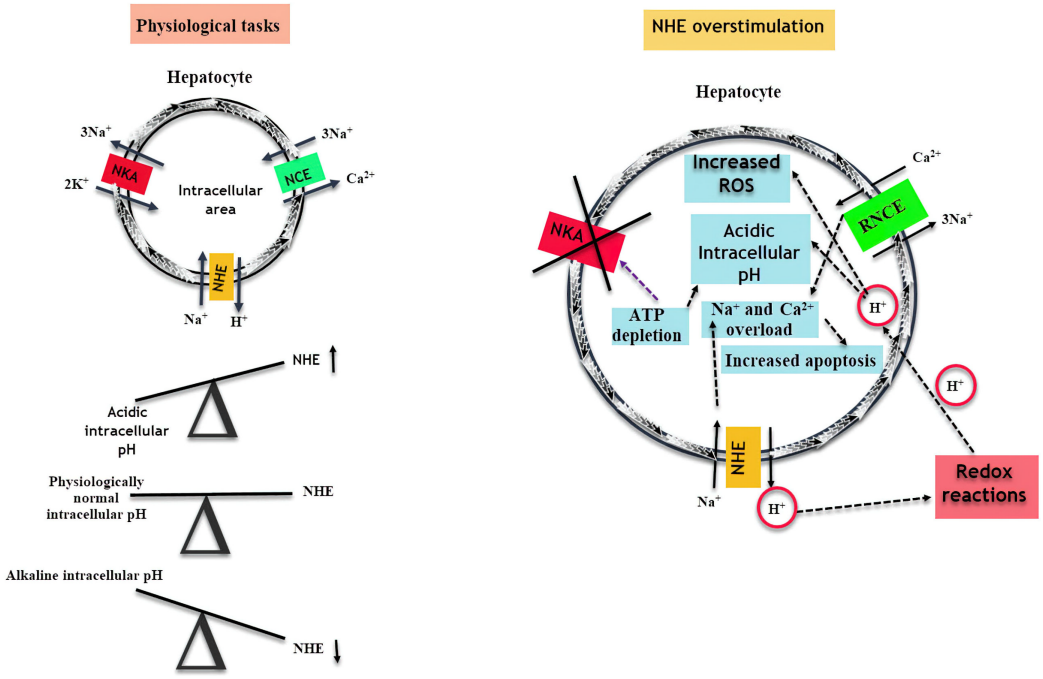
NHE is localized in the basolateral or sinusoidal membrane of hepatocytes[22]. The NHE, which provides the intracellular pH balance, is physiologically activated when the intracellular pH decreases and brings the intracellular pH to its physiological levels[25]. NHE causes the movement of Na^+ into the cell and H^+ out of the cell. As soon as the intracellular pH reaches physiological levels, the NHE activity reduces[12]. NHE provides the passage of Na^+ into the cell. The $Na^+/K^+/2Cl^-$ cotransporters cause the influx of Na^+, K^+, and Cl^- into the cell. Simultaneously, the $Cl^-/HCO3^-$ antiporter ensures the influx of Cl^- into the cell. Na^+/K^+-ATPase (NKA) pumps Na^+ outward while K^+ moves into the cell. Eventually, KCl increases in hepatocytes[25-27]. Na^+/Ca^{2+} Exchanger (NCE) provides intracellular Ca^{2+} balance[28]. These pumps work in harmony in the liver. Physiological NHE, NCE, and NKA activities are summarized in Figure 1.

In acute ischemic events, NHE activation increases intracellular pH and prevents hepatocytes from undergoing apoptosis[10]. Arginine vasopressin activates NHE in hepatocytes by calcium/calmodulin-dependent processes[29]. Stimulation of NHE is involved in hepatocyte regeneration and growth[25]. However, if the stimulus is increased, hepatocyte apoptosis due to intracellular Ca^{2+} accumulation increases[22,25].

Chronic cytokine or platelet-derived growth factor-mediated NHE stimulation causes hepatic stellate cell proliferation and fibrosis[30]. NHE3 is located in the apical membranes of the hepatocytes and cholangiocytes. NHE3 is responsible for maintaining the fluid content of bile acid[31]. Increased NHE3 activity leads to Na^+ loading and an increase in the concentration function of the gallbladder, resulting in gallstone formation[32-34].

PROLONGED NHE ACTIVATION

While NHE does significant work in physiological conditions, its continuous activation causes serious problems. During NHE physiological function, NCE pumps Na^+ into the cell and Ca^{2+} outward. As a result of NHE overstimulation, NCE activation stops, and reverse NCE becomes active due to increased Na^+ in the cell. While Ca^{2+} is pumped into the cell, Na^+ begins to be pumped outward. As a result, the concentration of Na^+ and Ca^{2+} in the cell increases. The NKA pump, which pumps K^+ inward and Na^+ outward, loses its function due to increased intracellular Na^+ and ATP depletion[4,12,35-37]. While edema occurs due to Na^+ accumulation in hepatocytes, Ca^{2+} overload causes hepatocyte apoptosis[38]. In the prolonged activity of NHE, continuous pumping of H^+ into the extracellular area causes some chain redox reactions, H^+ begins to influx into the cell, and the intracellular pH drops[4,12,29]. NHE overstimulation and its outcomes are summarized in Figure 1. NHE activation also increases the influx of lipids and free fatty acids into the cell. Free fatty acid influx further increases intracellular acidity, and concomitant Ca^{2+} overload accelerates apoptosis[36]. As a result of all these events, acute hepatocyte damage may progress from an advanced level to liver failure. Tanaka *et al*[39] found that NHE suppression inhibited the nuclear factor kappa B pathway and proinflammatory cytokine release, thus preventing fatal acute liver failure. In addition, there is a strong interaction between NHE and low-density lipoprotein receptors (LDLR), which may cause liver damage. Non-physiological LDLR excess gives rise to cholesterol overload in hepatocytes. Lipid deposition causes hepatomegaly in the chronic



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Figure 1 Na^+/H^+ exchanger overstimulation and its outcomes. Physiologically, the Na^+/H^+ exchanger (NHE) causes Na^+ to flow inward and H^+ to flow outward. When the intracellular pH falls, NHE is activated, raising the intracellular pH to its physiological level. NHE activity decreases as the intracellular pH increases. When NHE is overstimulated, increased Na^+ in the cell activates the reverse Na^+/Ca^{2+} exchanger and Ca^{2+} flows inward. The increased H^+ in the extracellular area causes chain redox reactions and an inwards H^+ influx. Reactive oxygen species increase, and ATP depletion inhibits Na+/K+-ATPase. As a result, hepatocytes are damaged and eventually die. NCE: Na^+/Ca^{2+} exchanger; NHE: Na^+/H^+ exchanger; NKA: Na^+/K^+-ATPase; RNCE: Reverse Na^+/Ca^{2+} exchanger; ROS: Reactive oxygen species.

process[40]. Although hepatocytes are resistant to cholesterol loading, hepatocytes begin to die with prolonged lipid deposition[40].

SARS-COV-2 AND NHE INTERACTION

The alkalinity of the intracellular pH creates a barrier against SARS-CoV-2 infection. Therefore, this mechanism has been considered for COVID-19 treatment[6]. Hydroxychloroquine has been used for COVID-19 treatment since it makes the intracellular pH alkaline by inhibiting vacuolar H^+-ATPase[41]. Also, metformin converts endosome pH to alkaline *via* vacuolar H^+-ATPase[42-44]. At or slightly above the physiological NHE activation can shift the intracellular pH to alkaline, preventing ACE2-SARS-CoV-2 fusion. Using proton pump inhibitors in mild COVID-19 patients may worsen the course of the disease[45,46]. However, when the virus takes total control of the cells in patients with severe COVID-19, NHE overstimulation causes many detrimental effects as well as a decrease in intracellular pH.

Hitherto, it has not been determined whether SARS-CoV-2 directly affects NHE; however, NHE activity was elevated in the blood of patients with COVID-19[11]. SARS-CoV-2 can increase NHE activity by many mechanisms. SARS-CoV-2-infected mice, which have physiological angiotensin II levels, have been shown to have no damage in some of their tissues[47]. However, patients with severe COVID-19 have high angiotensin II levels[48]. ACE2 degrades angiotensin II and reduces its levels. When SARS-CoV-2 fuses with ACE2, the level of angiotensin II increases in the circulation and tissues since ACE2 cannot complete its task[4]. The liver has a local renin-angiotensin system that is not very important in physiological conditions, which is dissimilar to hepatic renin-angiotensin system that plays a significant role in pathological conditions[49,50]. Since the liver expresses ACE2, the virus increases the local angiotensin II level by binding to ACE2 in the liver. Angiotensin II is the substantial stimulus of NHE. Angiotensin II shows pro-oxidant, fibrogenic, and proinflammatory actions on the liver[51].

SARS-CoV-2 uses lipid rafts and cholesterol to fuse with ACE2 in membranes[52]. Lipid rafts play a remarkable role in the entry of SARS-CoV-2 into the cell[53]. In addition, there is a strong interaction among lipid rafts, cholesterol, and NHE. Therefore, the virus can activate NHE through cholesterol and lipid rafts[36]. The release of proinflammatory cytokines such as tumor necrosis factor-alpha and interleukin-6 and increased oxidative stress by SARS-CoV-2 can also stimulate NHE[12]. Fibrinogen produced in the liver provides activation of NHE1 by inducing NCE[54]. Fibrinogen increase in COVID-19 can also initiate NHE activation.

SARS-COV-2 AND NHE IN THE LIVER

Since the liver has many vital functions, the virus infecting it will have many systemic consequences. Physiologically, NHE protects hepatocytes from apoptosis by making the intracellular pH alkaline[10]. However, NHE overstimulation can cause acute lethal liver damage[39]. Once the virus settles in the liver and infects hepatocytes, it can cause NHE overstimulation. The virus renders intracellular Ca^{2+} accumulation with reverse NCE activation after Na^+ accumulation in hepatocytes. Apoptosis is induced in hepatocytes and cell death. Liver enzymes such as AST and ALT increase due to parenchymal damage[55]. NHE overstimulation may also trigger a cytokine storm by increasing proinflammatory cytokines in the liver[12].

On the other hand, NHE1 plays a role in urea synthesis[56]. Increased H^+ extrusion increases urea synthesis[57]. The virus can increase the level of urea by NHE1 overstimulation. Increased urea in patients with COVID-19 indicates kidney damage[58]. However, urea may also be a marker of liver injury.

Gluconeogenesis in the liver is significantly reduced when the intracellular pH is lowered. Therefore, a hypoxic environment occurs[59,60]. In physiological conditions, NHE stimulates gluconeogenesis by increasing intracellular pH. NHE overstimulation decreases gluconeogenesis in the liver by reducing intracellular pH[61]. In patients with severe COVID-19, elevated blood glucose may be due to insulin resistance[61] or decreased insulin secretion related to NHE and proinflammatory cytokine-mediated pancreatic damage[62].

Other liver functions are vitamin B_{12} storage[63], iron storage[64], and vitamin D synthesis[65]. Vitamin D reduces acute liver injury[66]. Vitamin D is immunomodulatory, and low levels may worsen the progression of COVID-19[67,68]. Insulin-like growth factor-1 levels[69,70], which stimulate vitamin D synthesis in hepatocytes, was lower in COVID-19 patients[71]. While vitamin D increases circulating insulin-like growth factor-1 levels[72], insulin-like growth factor-1 inhibits NHE. Increased intracellular Ca^{2+} decreases vitamin D synthesis in the liver[73]. NHE-mediated hepatocyte injury in patients with COVID-19 may reduce vitamin D synthesis and worsen disease progression. Reduced intracellular pH leads to iron release from the liver[74], and excess iron causes local and systemic damage with the Fenton reaction[75]. Low intracellular pH caused by NHE overstimulation can lead to iron-mediated damage to cells in patients with COVID-19.

According to the LDLR mechanism we described earlier, the virus can cause lipid accumulation in the liver, leading to the fattening and enlargement of the liver and even loss of function[36]. Unfortunately, there is not enough information in the literature regarding the role of LDLR in patients with COVID-19. Although a study proved the opposite[76], Lange *et al*[77] reported that LDLR expression was higher in patients with COVID-19 than in healthy controls. Agirbasli *et al*[78] reported that LDLR-related protein 1, a member of the LDLR family, is increased in severe COVID-19. According to current findings, angiotensin II inhibits proprotein convertase subtilisin/kexin type 9[36], which disrupts LDLR[36]. Angiotensin II indirectly increases LDLR levels. In severe cases of COVID-19, increased angiotensin II may lead to NHE overstimulation and lipid accumulation in the liver. In addition, the virus needs cholesterol particles to form new virions after infecting cells[79]. Since the liver is the production and storage site of cholesterol, the virus can continue its life cycle in the liver for a lengthy time. Some studies have shown that high-density lipoprotein (HDL) binds to SARS-CoV-2[80]. Low HDL facilitates the virus binding to ACE2[80]; however, high HDL may play an active role in SARS-CoV-2 transfer from other infected tissues to the liver, as HDL is a cholesterol transporter of the liver.

The virus may settle in the liver and cause hepatocyte damage and multisystem disorder. Therefore, liver involvement may contribute significantly to mortality in COVID-19. There may be a close relationship between liver injury and fibrinogen and coagulation disorders in patients with COVID-19 [3]. As we know, fibrinolysis increases liver necrosis[81]. Initially, fibrinogen and D-dimer levels are elevated in patients with severe COVID-19, but their levels decrease over the following days[82]. Fibrinogen is an acute phase reactant primarily released from the liver, and interleukin-6 increases fibrinogen synthesis[83]. Since the release of proinflammatory cytokines such as interleukin-6 increases with NHE activation, the virus may indirectly cause an increase in fibrinogen and D-dimer. In severe cases of COVID-19, fibrinogen and D-dimer levels were low after 10 d.

NHE increases the tendency for thrombosis mainly through platelets[84]; however, it may also indirectly lead to a tendency for thrombosis through the liver. Prothrombin synthesized in the liver is converted to thrombin when fibrinogen is transformed into fibrin. Thrombin levels are high in patients with COVID-19[85]. Thrombin activates NHE in vascular smooth muscle cells[86]. Thus, the virus can cause damage to distant organs with the help of NHE-mediated proinflammatory cytokine release and thrombin.

While the virus causes a Na^+ load in the cell by NHE overstimulation, it overloads H^+ in the extracellular area[4]. As a result of the subsequent redox reactions, H^+ flows into the cell, and excessive ATP consumption by NKA results in hypoxia and ATP depletion in the cell[12]. Hypoxia and ATP depletion are prominent stimuli of heme oxidase[87,88]. Increased heme oxidase activation enhances carbon monoxide and bilirubin levels in the cell[89]. Carbon monoxide usually has a hepatoprotective effect, but sometimes it can also have a hepatotoxic effect[88]. Elevated bilirubin in patients with COVID-19 has been identified as a predictive marker for mortality rates[90]. Elevated bilirubin may

indirectly reflect NHE-mediated cell hypoxia in patients with COVID-19.

The elevation of alkaline phosphatase and GGT in COVID-19 patients suggests that SARS-CoV-2 changes the structure of bile acid and may lead to cholestasis[91]. NHE1 on the basolateral surface of the bile ducts regulates intracellular pH, while NHE3 on the apical surface regulates bile acid structure and fluidity[92]. Increasing NHE3 activity leads to Na^+ loading, which impairs the containment and fluidity of bile acid[32]. Bile acids play a primary role in maintaining gut microbiota composition[93]. We have recently described that disruption of NHE-mediated gut microbiota composition leads to cytokine release syndrome[12]. The virus disrupts the gut microbiota composition in the intestine and increases the release of proinflammatory cytokines like tumor necrosis factor-alpha. In addition, SARS-CoV-2 increases local angiotensin II levels by binding to ACE2. Unlike other tissues, tumor necrosis factor-alpha and angiotensin II decrease NHE3 activity in the intestine[12,94,95]. Thus, increased luminal Na^+ leads to diarrhea and cytokine release[12,96]. This event may play a role in triggering cytokine storms [12]. The virus can cause intestinal-associated cytokine release by changing the structure and fluidity of bile acid before it has intestinal involvement. It is not yet known whether the changed content of bile acid will play a role in transferring the virus to the intestine. Detailed studies are needed on this subject.

CONCLUSION

Physiological NHE activation protects the liver against acute injury, whereas NHE overstimulation causes severe liver injury. NHE overstimulation can lead to hepatocyte death. Also, it increases the urea cycle and inhibits of gluconeogenesis and vitamin D synthesis in the liver. NHE overstimulation may cause thrombotic events and systemic damage by increasing fibrinogen levels and cytokine release. It can also change the gut microbiota composition by disrupting the structure and fluidity of bile acid, thus triggering systemic damage. SARS-CoV-2-induced local and systemic damage can be improved by preventing virus-induced NHE overstimulation in the liver.

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MINIREVIEWS

Association between COVID-19 and chronic liver disease: Mechanism, diagnosis, damage, and treatment

Ruo-Bing Qi, Zheng-Hao Wu

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Abstract

As the outbreak evolves, our understanding of the consequences of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and the resulting coronavirus disease 2019 (COVID-19) on the liver has grown. In this review, we discussed the hepatotropic nature of SARS-CoV-2 and described the distribution of receptors for SARS-CoV-2 (*e.g.*, angiotensin-converting enzyme 2) in the vascular endothelium and cholangiocytes of the liver. Also, we proposed mechanisms for possible viral entry that mediate liver injury, such as liver fibrosis. Due to SARS-CoV-2-induced liver damage, many COVID-19 patients develop liver dysfunction, mainly characterized by moderately elevated serum aminotransferase levels. Patients with chronic liver disease (CLD), such as cirrhosis, hepatocellular carcinoma, nonalcoholic fatty liver disease, and viral hepatitis, are also sensitive to SARS-CoV-2 infection. We discussed the longer disease duration and higher mortality following SARS-CoV-2 infection in CLD patients. Correspondingly, relevant risk factors and possible mechanisms were proposed, including cirrhosis-related immune dysfunction and liver decompensation. Finally, we discussed the potential hepatotoxicity of COVID-19-related vaccines and drugs, which influence the treatment of CLD patients with SARS-CoV-2 infection. In addition, we suggested that COVID-19 vaccines in terms of immunogenicity, duration of protection, and long-term safety for CLD patients need to be further researched. The diagnosis and treatment for liver injury caused by COVID-19 were also analyzed in this review.

Key Words: SARS-CoV-2; COVID-19; Chronic liver disease; Angiotensin-converting enzyme 2; Hepatotoxicity; Calcineurin inhibitors

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Core Tip: In this review, we discussed the hepatotropic nature of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and described the distribution of receptors for SARS-CoV-2 in the vascular endothelium and cholangiocytes of the liver. We proposed mechanisms for possible viral entry that mediate liver injury, such as liver fibrosis. Due to SARS-CoV-2-induced liver damage, many coronavirus disease 2019 (COVID-19) patients develop liver dysfunction. We discussed the longer disease duration and higher mortality following SARS-CoV-2 infection in chronic liver disease patients. Correspondingly, relevant risk factors and possible mechanisms were proposed. Finally, we discussed the potential hepatotoxicity of COVID-19-related vaccines and drugs.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a disease caused by a novel coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). On December 31, 2019, the World Health Organization first learned about this new virus from a set of cases of viral pneumonia reported in Wuhan, People's Republic of China. The most common symptoms of COVID-19 are fever, dry cough, and fatigue. In particular, symptoms of severe COVID-19 often present with dyspnea, loss of appetite, confusion, and high fever. Of those who develop symptoms, the majority (about 80%) do not require hospitalization to recover. About 15% of patients are severely ill and require oxygen; 5% of patients are critically ill and require intensive care.

Complications of death from COVID-19 may include respiratory failure, acute respiratory distress syndrome, sepsis and septic shock, thromboembolism, and/or multiple organ failure, including heart, liver, or kidney damage. In particular, people aged 60 and older, as well as those with underlying medical conditions such as high blood pressure, cardiorespiratory problems, diabetes, obesity, or cancer, are at higher risk of developing severe COVID-19.

Currently, individual COVID-19 vaccines have been licensed for use by regulatory agencies in some countries, and many potential COVID-19 vaccine candidates are under development. This article analyzed and summarized COVID-19 from four aspects: Mechanism, diagnosis, damage, and treatment. Table 1 summarizes the analysis of these four parts.

MECHANISM

Our understanding of the hepatic consequences of SARS-CoV-2 infection and the resulting COVID-19 has evolved rapidly since the beginning of the pandemic[1]. Many reports showed that many COVID-19 patients had chronic liver disease (CLD) of varying degrees[2-5]. In particular, COVID-19-related liver injury refers to any liver injury that occurs in patients with COVID-19 during the course and treatment of the disease, regardless of pre-existing liver disease[6-8].

Several studies have shown that SARS-CoV-2 can bind to the host angiotensin-converting enzyme 2 (ACE2) receptor, allowing the virus to enter cells and actively replicate in the liver[9-11]. Notably, severe disease outcomes depend on the high affinity of the virus to ACE2[12]. ACE2 is expressed in multiple organs, such as the lung, gastrointestinal tract, and liver[13-15]. In the liver, ACE2 is expressed at a low level in hepatocytes, with a positive rate as low as 2.6%[16]. However, it is highly enriched (59.7%) in cholangiocytes, similar to the expression levels in the type II alveolar cells (primary target cells of SARS-CoV-2 in the lung)[17,18]. Therefore, the virus may directly infect bile duct cells but not hepatocytes[13]. Viral infection could lead to cholangiocyte apoptosis accompanied by mitochondrial swelling, endoplasmic reticulum expansion, reduction of glycogen granules, and extensive necrosis[19, 20].

SARS-CoV-2 infection can lead to severe host hyperimmunity in the lungs, triggering a life-threatening cytokine storm[21,22], a systemic inflammatory response syndrome driven by viral infection. Cytokine storm syndrome may induce a massive release of multiple proinflammatory cytokines and inflammatory markers[23], leading to tissue damage and multiple organ damage or failure, including the liver[24]. Cytokine storms caused by virus-induced excessive immune response may be one of the pathways of CLD[25,26].

Table 1 Summary of the mechanism, diagnosis, damage, and treatment of coronavirus disease 2019 in chronic liver disease patients

Feature	Conclusion of each part
Mechanism	SARS-CoV-2 can bind to the host ACE2 receptor, allowing the virus to enter cells and actively replicate in the liver. Severe disease outcomes depend on the high affinity of the virus to ACE2. In addition, SARS-CoV-2 infection can lead to severe host hyperimmunity in the lungs, triggering a life-threatening cytokine storm[21,22], a systemic inflammatory response syndrome driven by viral infection. This leads to tissue damage and multiple organ damage or failure. In addition, symptoms due to COVID-19 complications are underlying pathological mechanisms of extensive liver injury
Diagnosis	Liver biochemical abnormalities are common in COVID-19-related CLD patients. The main manifestations of patients with COVID-19-related CLDs are moderately elevated serum transaminase activity and elevated LDH levels. The severity of CLD during the COVID-19 course can be effectively judged by detecting serum transaminase, LDH, bilirubin levels, and albumin concentrations
Damage	Invasion of SARS-CoV-2 may lead to significant systemic disease; some can even develop severe lung disease, leading to respiratory compromise, which in turn may progress to multiple organ failure, coagulopathy, and death. Typically, COVID-19 patients are more vulnerable and susceptible to underlying metabolic diseases. In addition, SARS-CoV-2 can cause CLD by direct cytopathies, immune-mediated, hypoxia/ischemia, and microvascular thrombosis
Treatment	Immunosuppression therapy is meaningful for both COVID-19 and CLD. Therefore, immunosuppressive drugs should be evaluated during the co-occurrence of both disorders Common immunosuppressive drugs include calcineurin inhibitors and mTOR inhibitors. Medication side effects need to be considered during treatment, including increasing susceptibility to SARS-CoV-2 infection and secondary bacterial or fungal infection and prolonged viral clearance. In addition, currently prescribed drugs for COVID-19 are all metabolized in the liver, and these antiviral drugs may lead to abnormal liver function. Therefore, it is necessary to balance the management of immunosuppressive therapy and drug interactions in patients with CLD infected with COVID-19 and minimize the use and dosage of immunosuppressants to reduce the impact of liver damage

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; ACE2: Angiotensin-converting enzyme 2; COVID-19: Coronavirus disease 2019; CLD: Chronic liver disease; LDH: Lactate dehydrogenase.

In addition, liver disease worsens because of COVID-19 complications, including coagulation disorders and cardiac and respiratory failure. These complications induced diffuse intravascular coagulation, ischemia, and hypoxia in the liver. All of these can lead to upregulation of fibrotic pathways, fatty acid oxidation, oxidative phosphorylation, and dysregulation of markers of immune activation. These are also the potential pathological mechanisms of extensive liver injury[27].

DIAGNOSIS

Liver biochemical abnormalities are common in COVID-19-related CLD patients, occurring in approximately 15%-65% of SARS-CoV-2 infected individuals[28-31]. The main manifestations of patients with COVID-19-related CLDs are moderately elevated serum transaminase activity and elevated lactate dehydrogenase (LDH) levels[32-35]. However, COVID-19 may cause damage to other organs, including the heart, bones, and kidneys. Skeletal muscle and myocardial injury can also lead to elevated serum transaminases and LDH levels[12,16]. In addition, hypoalbuminemia was reported to be a nonspecific marker of disease severity associated with poor COVID-19 prognosis[36]. Therefore, the severity of CLD during the COVID-19 course can be effectively judged by detecting serum transaminase, LDH, bilirubin levels, and albumin concentrations[37-40].

DAMAGE

Invasion of SARS-CoV-2 may lead to significant systemic disease involving the gastrointestinal tract, liver, biliary tract, and pancreas[12]. Most patients with SARS-CoV-2 infection are asymptomatic or have mild symptoms, including fever, cough, loss of smell, and headache[1]. However, approximately 15% of patients develop severe lung disease within 10 d, leading to respiratory compromise, which in turn may progress to multiple organ failure, coagulopathy, and death[41-43].

COVID-19 patients are more vulnerable and susceptible to underlying metabolic diseases, including hypertension, cardiovascular disease, type 2 diabetes, chronic lung disease, and metabolic syndrome[44,45]. In particular, people at high risk for severe COVID-19 are usually the elderly and those with comorbidities[37]. In addition, obese patients who frequently develop the metabolic dysfunction-associated fatty liver disease are also at high risk of developing severe COVID-19 due to the role of acutely active inflammatory pathways[19,38]. Infection with SARS-CoV-2 can increase the severity of viral hepatitis, and its clearance in patients is delayed. For those underlying undetected liver diseases, especially nonalcoholic fatty liver disease and cirrhosis, the prevalence of COVID-19 is significantly increased, and the prognosis will be worse[46]. For CLD patients, especially those with advanced liver disease, SARS-CoV-2 infection may seriously jeopardize survival and exacerbate liver failure in the case of the diminished liver reserve[47,48].

In conclusion, SARS-CoV-2 can cause CLD in the following aspects: Direct cytopathies (SARS-CoV-2 invades liver cells and causes cytopathic effects leading to liver dysfunction in COVID-19 patients); immune-mediated (SARS-CoV-2 infection leads to a disordered inflammatory response and increased proinflammatory cytokines, which in turn triggers severe liver dysfunction); hypoxia/ischemia (in severe COVID-19, multiple organ dysfunction can lead to hypoxia-related acute respiratory distress syndrome[49], hypotension[50], or congestive heart failure, which in turn leads to liver dysfunction); and microvascular thrombosis.

TREATMENT

CLD is common worldwide. The rapid spread of COVID-19 has resulted in infections in many patients with underlying CLD. Immunosuppression therapy is meaningful for both COVID-19 and CLD. Therefore, immunosuppressive drugs should be evaluated during the co-occurrence of both disorders. First, calcineurin inhibitors (CNIs), including cyclosporine or tacrolimus, are considered the basic drugs for immunosuppression in treating CLD[51]. They are often used with mycophenolate mofetil or everolimus to reduce plasma levels. Their use avoids the adverse effects of cyclosporin A binding to the intracellular receptor cyclosporine to form an active complex. This may inhibit the phosphatase activity of calcineurin. Second, immunosuppressants such as mycophenolate mofetil and CNIs have been shown to have antiviral activity against coronaviruses[52]. There is evidence that CNIs have direct antiviral effects. Cyclosporine can block replication of all coronavirus genera, including SARS-CoV. Similarly, mTOR inhibitors (*e.g.*, tacrolimus) have antiviral properties in addition to their immunosuppressive and antiproliferative effects. Glucocorticoids for COVID-19 have been shown to prevent the disturbances in the immune response that lead to the poor prognosis of COVID-19[53].

The side effects cannot be ignored, despite the indispensable role of immunosuppression therapy in COVID-19-related CLD. Immunosuppression induced by these drugs may increase susceptibility to SARS-CoV-2 infection[54] and secondary bacterial or fungal infection. In addition, it may also prolong viral clearance time[55]. Related research shows that patients using immunosuppressive drugs have an increased average risk of SARS-CoV-2 infection. Therefore, experience suggests that reducing mycophenolate mofetil or mTOR inhibitors remains beneficial for managing immunosuppression during SARS-CoV-2 infection. Patients who received thiopurines and glucocorticoids before the onset of COVID-19 had a higher risk of severe COVID-19 than CLD patients who were not receiving immunosuppressive therapy[56]. In particular, patients with severe COVID-19 infection may need to consider dose adjustment of steroids, CNIs, or mycophenolate mofetil to reduce the effect of liver injury.

In addition, currently prescribed drugs for COVID-19 (*e.g.*, oseltamivir, lopinavir/ritonavir, and chloroquine) are all metabolized in the liver. Although there is currently no recognized effective antiviral drug for COVID-19, nearly half of the critically ill patients were prescribed antiviral drugs such as oseltamivir, abidol, lopinavir, and ritonavir[57]. These antiviral drugs may cause abnormal liver function. In particular, patients with CLD, such as hepatitis B or C, may have elevated aminotransferase levels before treatment, which may increase the risk of drug-induced liver injury[46]. Therefore, attention should be paid to abnormal liver test indicators during the treatment process to reduce drug-induced liver injury[58].

In summary, the management of immunosuppressive therapy and drug interactions in patients with CLD infected with COVID-19 must be balanced. The effects of drugs on liver toxicity, steatosis, necroinflammation, fibrosis, and biological metabolism should be comprehensively considered when treating COVID-19. This is beneficial to avoiding serious drug-induced liver injury while exerting a sufficient immune response and antiviral effect[12].

Finally, although these patients have compromised immune responses, immediate and long-term protective responses through immunization may not be complete for the protective measure of vaccination. However, early vaccination against various pathogens, including SARS-CoV-2 in patients with CLD remains essential and effective[59]. A small number of patients have mild jaundice (slightly elevated bilirubin levels).

CONCLUSION

Above all, SARS-CoV-2 can bind to the host ACE2 receptor, allowing the virus to enter cells and actively replicate in the liver. Severe disease outcomes depend on the high affinity of the virus to ACE2. SARS-CoV-2 infection can lead to severe host hyperimmunity in the lungs, triggering a life-threatening cytokine storm[21,22], a systemic inflammatory response syndrome driven by viral infection. This leads to tissue damage and multiple organ damage or failure. In addition, symptoms due to COVID-19 complications are also underlying pathological mechanisms of extensive liver injury.

Liver biochemical abnormalities are common in COVID-19-related CLD patients. The main manifestations of patients with COVID-19-related CLDs are moderately elevated serum transaminase activity and elevated LDH levels. The severity of CLD during the COVID-19 course can be effectively judged by

detecting serum transaminase, LDH, bilirubin levels, and albumin concentrations.

Invasion of SARS-CoV-2 may lead to significant systemic disease, and some patients can even develop severe lung disease, leading to respiratory compromise, which in turn may progress to multiple organ failure, coagulopathy, and death. Typically, COVID-19 patients are more vulnerable and susceptible to underlying metabolic diseases. SARS-CoV-2 can cause CLD by direct cytopathies, immune-mediated, hypoxia/ischemia, and microvascular thrombosis.

Immunosuppression therapy is meaningful for both COVID-19 and CLD. Therefore, immunosuppressive drugs should be evaluated during the co-occurrence of both disorders. Common immunosuppressive drugs include CNIs and mTOR inhibitors. Medication side effects need to be considered during treatment, including increasing susceptibility to SARS-CoV-2 infection and secondary bacterial or fungal infection and prolonged viral clearance. In addition, currently prescribed drugs for COVID-19 are all metabolized in the liver, and these antiviral drugs may lead to abnormal liver function. Therefore, it is necessary to balance the management of immunosuppressive therapy and drug interactions in patients with CLD infected with COVID-19 and minimize the use and dosage of immunosuppressants to reduce the impact of liver damage.

FOOTNOTES

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MINIREVIEWS

COVID-19 in patients with pre-existing chronic liver disease – predictors of outcomes

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Abstract

Coronavirus disease 2019 (COVID-19) has affected patients with pre-existing chronic liver disease (CLD) in various ways. The maximum impact was seen on patients with underlying cirrhosis who have shown to have poor clinical outcomes in the form of increased risk of hepatic decompensation, acute-on-chronic liver failure, and even mortality. It is of paramount importance to identify various factors which are associated with unfavorable outcomes for prognostication and making informed management strategy. Many factors have been evaluated in different studies in patients with underlying CLD. Some of these factors include the severity of underlying chronic liver disease, comorbid conditions, age, and severity of COVID-19. Overall, the outcomes are not favorable in patients with cirrhosis as evidenced by data from various studies. The main purpose of this review is to identify the predictors of adverse clinical outcomes including mortality in patients with CLD for risk stratification, prognostication, and appropriate clinical management.

Key Words: Severe acute respiratory syndrome coronavirus 2; Cirrhosis; Predictors; Outcomes

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Core Tip: Coronavirus disease 2019 (COVID-19) has been shown to negatively affect the outcomes of patients with chronic liver disease. Some of the major factors predicting poor outcomes and mortality as shown by various studies include old age (> 60 years) and presence of comorbidities like diabetes, hypertension, and obesity. Apart from these, the most important outcome measure is the severity of underlying chronic liver disease and in some cases the etiology of chronic liver disease. Another major predictor of outcome is the severity of COVID-19, with respiratory failure being a common cause of mortality. Further data is required to draw a definitive relation between these risk factors and outcomes in these patients.

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INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–related coronavirus disease 2019 (COVID-19) has wreaked havoc since its outbreak in late 2019. As per the most recent estimates, it has affected more than 575 million people worldwide[1]. It has adversely affected the health care system even in developed nations. Although the most common manifestations of COVID-19 are either asymptomatic infection or mildly symptomatic infection with fever, cough, and generalized weakness, some patients developed severe respiratory failure requiring mechanical ventilation and even death[2].

Patients with chronic liver disease (CLD) have been affected due to the COVID-19 pandemic, such as lack of routine services like variceal and hepatocellular carcinoma (HCC) screening, lack of physical follow-up to monitor the response to treatment like ascites, re-allocation of health care facilities for COVID-19 management, *etc.* An acute insult in the form of COVID-19 in a background of CLD may lead to further decompensation and increased morbidity and mortality. Therefore, in this review, we will summarize and identify the predictors of adverse outcomes in patients with CLD, which will help in prognostication, risk stratifying, and providing optimal care to the patients.

EPIDEMIOLOGY

COVID-19 is a systemic disease affecting multiple organ systems and gastrointestinal system (GI) involvement may be seen in a subset of patients. Studies have shown that nearly 20% of the affected patients had some abnormalities in liver function as reflected by elevated liver enzymes (20%) and elevated bilirubin (16%)[3]. About 35% of patients showed abnormal alanine aminotransferase or bilirubin levels, out of which 77% showed elevation to levels less than 5 times the upper limit of normal [4]. The prevalence of underlying CLD in COVID-19 patients was 3%-6.3% in various studies[5-8]. A meta-analysis of 73 studies with 24299 patients, showed that the prevalence of CLD in COVID-19 positive patients was 3%, which was similar to that among COVID-19 negative patients[3]. The differences in prevalence might be due to admission bias, sampling bias, and retrospective nature of studies.

A recent study showed that mortality rates among alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) affected patients increased significantly during the pandemic while the rate of mortality among patients with viral hepatitis remained similar to the pre-pandemic times[9]. A recent meta-analysis of 40 studies has shown that patients with CLD had a significantly higher risk of severe COVID-19 (pooled odds ratio [OR]: 2.44) and death (pooled OR: 2.35) as compared to COVID-19 patients without[10]. Hence, COVID-19 does affect patients with CLD and in some cases with adverse outcomes.

PATHOPHYSIOLOGY OF LIVER INVOLVEMENT IN COVID-19

The liver involvement in COVID-19 is multifactorial, including direct viral hepatotoxicity, immune-mediated liver injury, sepsis, hypoxemia, or drug-induced liver injury[11]. Direct hepatotoxicity is due to entry of SARS-CoV-2 into the liver through the binding of viral spike (S) protein to the angiotensin-converting enzyme 2 (ACE2) receptor on cholangiocytes. The expression of ACE2 is highest in cholangiocytes followed by sinusoidal endothelial cells and hepatocytes as shown in healthy livers by single cell RNA sequencing methods[12]. After entering the cell, the S protein is primed by a specialized serine

protease, transmembrane serine protease 2 (TMPRSS2), in the host cell[13]. Once inside the cell, SARS-CoV-2 causes activation of the mTOR pathway which inhibits autophagy of the viral particles. Thus, the viral particles evade the immune system and increase in number, exerting direct hepatotoxicity *via* mitochondrial dysfunction, ER stress, and activation of the intrinsic pathway of apoptosis as depicted in Figure 1[14].

The second hit in the pathogenesis of liver injury is secondary damage caused by cytokine storm. Infection with SARS-CoV-2 has been postulated to cause a massive surge in proinflammatory cytokine levels. The predominant cytokines implicated include interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, and elevated levels of ferritin and CRP[15]. These proinflammatory cytokines result in cholestatic liver injury by causing downregulation of proteins and channels involved in uptake and secretion of bilirubin and bile salts similar to what is seen in sepsis-related cholestasis. Another postulated mechanism is decreased albumin synthesis due to IL-6 mediated suppression of C/EBP pathway[14]. TNF-α and IL-1 also activate and recruit macrophages to the liver and induce apoptosis of hepatocytes. These inflammatory cytokines also cause hypoxic liver injury by causing endothelial damage and inducing microvascular thrombosis[14]. Thus, various cytokines act in concert to cause liver injury reflected as biochemical alterations in liver function. Other mechanisms include hypoxemic injury secondary to type 1 hypoxemic respiratory failure and drug induced liver injury.

CHRONIC LIVER DISEASE AND COVID-19 OUTCOMES

Predictors of outcomes in chronic liver disease

Numerous studies across the globe have tried to identify the predisposing factors for poor outcomes of COVID-19 in patients with CLD as summarized in Figure 2 and Table 1. A summary of evidence available and the possible risk factors has been enumerated below.

Age: Increasing age is associated with a blunted immune response and multiple comorbidities, and thus may have an impact on the outcomes in patients with pre-existing CLD. Age more than 60 years had an adjusted hazard ratio (aHR) of 1.05 for mortality in cirrhotics with COVID-19[16]. Another study showed that the mortality increased from 6.1% in patients aged less than 50 years to 33.9% among those who were more than 65 years with an adjusted OR (aOR) of 7.2 in this group of patients[17]. Therefore, increasing age especially more than 60 years has been associated with increased mortality and risk of liver decompensation[16-18].

Ethnicity: African Americans with CLD were twice more likely to develop COVID-19 than Caucasians in a study[19]. Another study found that non-Hispanic blacks and Hispanics had higher chances of contracting COVID-19 in patients with CLD; however, they did not find any difference in outcomes of disease in different ethnicities[20]. Hispanics had more severe COVID-19 infection in patients with CLD [18]. Henceforth, Hispanics and blacks have been shown to have higher risk of contracting COVID-19 disease and having a severe course likely due to lower socioeconomic status, poverty, overcrowding, and inadequate access to health care services.

Etiology of CLD: Several studies have tried to relate the etiology of CLD with outcomes in COVID-19 patients. In the following sections, the determinants of outcomes are described according to etiology of CLD.

Alcohol

Alcohol is one of the most common etiologies of CLD. The pandemic led to a situation of social isolation and unemployment which lead to an increased consumption of alcohol in higher quantities[21]. A study found a higher rate of mortality among alcohol-related liver disease with an aHR of 1.79[22]. Kim *et al* [18] showed an aHR of 2.42 of mortality among alcohol-related liver disease. There was a three times increase in the monthly percent change of crude ALD-related mortality after February 2020 as compared to January 2017 to December 2017 in one study[23]. However, another study did not find alcohol as a poor outcome variable on multivariate analysis of retrospective data[24,25]. A recent study showed that the mortality among ALD patients was declining in the pre-pandemic era but increased fivefold during the pandemic[9]. Therefore, it seems that alcohol as an etiology increases the risk of adverse outcomes and mortality[26] in patients with COVID-19 and CLD as summarized in Table 2 and Supplementary Table 1.

NAFLD

NAFLD is rapidly becoming the most common cause of CLD across the world. It is considered to be the hepatic manifestation of metabolic syndrome and usually coexists with other components of metabolic syndrome. The COVID-19 pandemic showed a bidirectional relationship with NAFLD. Lockdown during the pandemic and lack of exercise lead to an increase in sedentary behavior and thus metabolic syndrome including NAFLD. Such patients had a more severe COVID-19 infection as evidenced by higher requirement of oxygen, mechanical ventilation, and prolonged intensive care unit (ICU) stay[27-

Table 1 Studies showing clinical outcomes of chronic liver disease in coronavirus disease 2019 and associated risk factors

Ref.	Type	Clinical outcomes	Predictors of outcomes
Iavarone *et al* [53]	Multicentric retrospective study of 50 cirrhotics	ACLF and *de novo* acute liver injury: 28%; 30-d mortality: 34%	Predictors of mortality: CLIF-OF (HR: 1.426); Moderate/severe respiratory failure (HR: 1.608)
Marjot *et al*[22]	Retrospective data from United Kingdom hospital network including 745 patients with CLD (386 with and 359 without cirrhosis)	Acute hepatic decompensation: 46%; ACLF: 50%; Mortality in cirrhosis, ACLF, and non-cirrhotics: 32%, 65%, and 8%	Predictors of mortality: ALD (OR: 1.79); Child-Pugh class: Child-Pugh A +2.0%, Child-Pugh B +20.0%, Child-Pugh C +38.1%. Predictors of decompensation: Child-Pugh class
Ge *et al*[16]	Data from the National COVID Cohort Collaborative (N3C) dataset of 6.4 million cases	3.31 times adjusted hazard of death in cirrhotics at 30 d than non-cirrhotics	Predictors of 30-d mortality: Age (aHR: 1.05 per year); Hispanic ethnicity (aHR: 1.20); Chronic hepatitis C (aHR: 1.27); ALD (aHR: 1.40); Modified CCI (aHR: 1.07 per point)
Elhence *et al*[24]	Retrospective analysis of 221 cirrhosis patients	Compensated cirrhosis: 8.1%; Acute decompensation: 62.9%; ACLF: 29.0%; MODS: 55.6%; Type 1 respiratory failure: 20.0%; Sudden cardiac arrest: 6.7%; GI bleeding: 3.3%	Predictors of mortality: Higher TLC [HR: 1.054]; Elevated creatinine [HR: 1.184]; MELD score [HR: 1.038]; Alkaline phosphatase [HR: 1.003]; COVID-19 severity [HR: 2.573]; ACLF on presentation (HR: 2.573)
Xiao *et al*[52]	Medical records collected from 23 Chinese hospitals	Decompensated cirrhosis: 57.5%; Mortality: 28.9%	Factors associated with mortality: Child-Pugh class (OR: 5.71); CURB65 (OR: 5.88)
Grgurevic *et al* [48]	4014 patients	Four times higher risk of 30-d mortality in cirrhosis	Predictor of 30-d mortality: Cirrhosis (HR: 2.95)
Mendizabal *et al* [17]	Prospective cohort of 96 cirrhosis patients	Mortality in cirrhotic: 47% *vs* 16% in non-cirrhotics; Acute decompensation: 61.4%; ACLF: 55.2%	Factors associated with mortality: Age > 65 yr (OR: 7.2); Male gender (OR: 1.8); BMI > 30 (OR: 1.7); Cirrhosis (OR: 3.1)
Kim *et al*[18]	Multicentre observational cohort study in 21 institutes in United States with 867 CLD cases (227 with cirrhosis)	Mortality: 25%; Hepatic decompensation: 7.7%; Hepatic encephalopathy: 34.3%; Ascites: 16.4%; Variceal bleed: 10.4%	Predictors of all-cause mortality: ALD (HR: 2.42); Hepatic decompensation at baseline (HR: 2.91); HCC (HR: 3.31); Increasing age (HR:1.44 per 10 yr); Diabetes (HR: 1.59); Hypertension (HR:1.77); COPD (HR:1.77); Current smoking (HR: 2.48)
Sarin *et al*[37]	Retrospective data from 13 Asian countries with228 patients [185 CLD without cirrhosis and 43 with cirrhosis]	ACLF: 11.6%; Acute decompensation: 9%; Mortality rate: 43% among decompensated cirrhotics	Predictors of sever liver injury: In CLD without cirrhosis, diabetes [57.7% *vs* 39.7%, OR: 2.1 (1.1-3.7)]; In cirrhotics, obesity [64.3% *vs* 17.2%, OR: 8.1 (1.9-38.8). Predictor of mortality: CTP score of 9 or more at presentation [AUROC 0.94, HR:19.2]
Xiang *et al*[54]	Retrospective cohort study of 267 patients	Severe COVID-19: 15%; High-flow oxygen support: 14%; Mechanical ventilator support: 4%; Death: 1	Predictor of severity: FIB-4 > 3.25

ACLF: Acute-on-chronic liver failure; ALD: Alcohol related liver disease; AUROC: Area under the receiver operator curve; BMI: Body mass index; COVID: Coronavirus disease; CLD: Chronic liver disease; CCI: Charlson's comorbidity index; CLIF OF: Chronic liver failure consortium organ failure; CLIF-C: Chronic liver failure consortium; COPD: Chronic obstructive pulmonary disease; CTP: Child-Turcotte-Pugh score; FIB-4: Fibrosis 4; HCC: Hepatocellular carcinoma; MELD Na: Model for end stage liver disease sodium score; MODS: Multiorgan dysfunction syndrome; NACSELD: North American Consortium for the Study of End-Stage Liver Disease.

Table 2 Clinical outcomes in patients with underlying alcoholic liver disease during coronavirus disease 2019

Ref.	Study	Outcomes
Deutsch-Link *et al*[23]	Retrospective analysis - pre (January 2017 to December 2017) and post-COVID era (February 2020)	Increase in the monthly percent change of crude ALD-related mortality: Males: 3.18 *vs* 0.96; Females: 3.8 *vs* 1.18
Yeo *et al*[26]	16813 patients with ALD before and 11625 during the pandemic	OR of death in ALD - 18.7 during the pandemic *vs* 0.995 in the pre-pandemic era

ALD: Alcohol related liver disease; COVID: Coronavirus disease; LT: Liver transplant; NASH: Non-alcoholic fatty liver disease; OR: Odds ratio; UNOS: United network for organ sharing.

30] (Table 3). Studies have demonstrated that patients with features of metabolic syndrome including higher body mass index (BMI) and waist circumference and presence of diabetes and hypertension had adverse effects on outcomes[29-31].

Patients with COVID-19 and underlying NAFLD have multiple associated comorbidities including diabetes, hypertension, dyslipidemia, and obesity. These factors have been independently associated with poor outcomes in patients with COVID-19[32,33]. Different studies have estimated different

Table 3 Studies evaluating outcomes and predictors of severity in non-alcoholic fatty liver disease with coronavirus disease 2019

Ref.	Type of study	Patients included	Outcomes	Predictors
Chang *et al* [27]	Retrospective study	3122 COVID-19 cases [FLI (fatty liver index) was calculated]	Severe disease: 223 (7.14%); Mechanical ventilation: 82 (2.63%); ICU admission: 126 (4.04%) High-flow oxygen therapy: 75 (2.40%); Death: 94 (3.01%)	FLI associated with severe complications from COVID-19 (aOR: 1.77)
Vrsaljko *et al*[28]	Prospective observational study	120 NAFLD patients (of 216 COVID-19 patients)	Patients with NAFLD had more high-flow nasal cannula or non-invasive ventilation (21.66%, *vs* 10.42%), longer duration of hospitalization (10 d *vs* 9 d), and more pulmonary thromboembolism risk (26.66% *vs* 13.54%)	Delayed time to recovery (HR: 0.64); Increased pulmonary thrombosis (OR: 2.15) among NAFLD patients
Velazquez *et al*[29]	Retrospective cohort study	359 NAFLD patients as per Dallas steatosis index (DSI) out of total 470 cases	Lower oxygen saturation levels; Higher D-dimer; Elevated LDH; Higher lymphocyte count among NAFLD	On multivariable analysis, NAFLD is a predictor of mortality (OR: 2.13)
Madan *et al* [36]	Retrospective observational case control study	289 NAFLD patients among 446 cases	Similar in-hospital mortality, ICU requirement, ventilatory support, and duration of ICU and hospital stay	Predictors of in-hospital mortality: High total leukocyte count (OR: 1.082); High FIB-4 (OR: 1.606)
Chen *et al* [34]	Retrospective single centre cohort study	172 patients with hepatic steatosis (HS) among 342 cases	19% of patients expired; > 50% required ICU admission	Increased intubation (aOR: 2.75); Vasopressor requirements (aOR: 1.22); ALT > 5 x ULN (aOR: 7.09)
Sarin *et al* [37]	Retrospective multinational cohort	113 NAFLD cases out of 228 cases (185 without cirrhosis and 43 with cirrhosis)	Higher risk of acute liver injury in obese cirrhotics *vs* normal weight patients (OR: 8.9)	Higher risk of liver injury: In non-cirrhotics, diabetes [57.7% *vs* 39.7%, OR: 2.1]; In cirrhotics, obesity, [64.3% *vs* 17.2%, OR: 8.1]
Li *et al*[31]	Observational study	Genome-wide meta-analysis (GWMA) of 3711 NAFLD cases and 426252 controls from United Kingdom Biobank data	No significant association of NAFLD and severe COVID-19 after adjusting for confounders	Predictors of severity: Body mass index (OR: 1.73); Waist circumference (OR: 1.76); Hip circumference (OR: 1.33)
Yao *et al* [30]	Retrospective study in China	86 COVID-19 patients with NAFLD	NAFLD patients with advanced fibrosis (NFS > -1.5) had more fever (81.6% *vs* 50%), shortness of breath (18.4% *vs* 0%), and severe disease (28.9% *vs* 2.1)	Predictors of severe disease: Diabetes (OR: 8.264); Advanced liver fibrosis [NFS > -1.5] (OR: 11.057)
Targher *et al*[35]	Retrospective study	94 NAFLD cases among 310 patients		Factors associated with severity: Increasing FIB-4 (aOR: 1.90); Increasing NFS (aOR: 2.57)

aOR: Adjusted odds ratio; ALT: Alanine transaminase; AST: Aspartate aminotransferase; COVID: Coronavirus disease; CLD: Chronic liver disease; FIB-4: Fibrosis 4; NFS: NAFLD fibrosis score; FLI: Fatty liver index; GWAS: Genome wide association studies; ICU: Intensive care unit; LDH: Lactate dehydrogenase; NAFLD: Non-alcoholic fatty liver disease; TLC: Total leukocyte count; ULN: Upper limit of normal.

prevalence of comorbidities in NAFLD. The prevalence of obesity, diabetes mellitus, and hypertension was 47%, 27%, and 31%, respectively, in NAFLD patients in a study, in which 27% of patients required non-invasive mechanical ventilation, 44% required ICU admission, and 27% were expired[29]. Another study showed that 69% of patients had hypertension, 43% had diabetes, 47% had dyslipidemia, 85% were overweight, and 52% were obese, and out of total 342 patients, > 50% required ICU admission and 19% were expired[34]. Thus, the presence of comorbidities is associated with poor outcomes in patients with NAFLD and COVID-19.

Advanced fibrosis in patients with NAFLD was associated with more severe COVID-19 and adverse outcomes with an almost two-fold increased risk of severity in patients with an FIB-4 score of more than 2.67[35,36]. Another study showed that presence of cirrhosis with diabetes was associated with poor outcomes in COVID-19 patients with higher risk of liver injury (OR: 2) and NAFLD was the most common cause of CLD in this study[37]. The rate of severe illness was significantly higher in patients with advanced fibrosis [NAFLD fibrosis score (NFS) > -1.5] compared to those with non-advanced fibrosis with an NFS < -1.5 (28.9% *vs* 2.1%, $P < 0.001$)[30]. Therefore, the underlying degree of fibrosis in NAFLD patients and various components of metabolic syndrome has been associated with poor outcomes as compared to those without significant fibrosis.

Hepatitis B

Patients with chronic hepatitis B (CHB) had a higher rate of ICU admission (HR: 1.86) and increase risk of mortality (HR: 3.19) among hepatitis B virus "e" antigen positive (HBeAg+) CHB cases as shown in Table 4[8,38,39]. Another study showed that although the mortality rate was higher among patients with hepatitis B, it was not statistically significant after adjusting for other factors[40]. The study had shown that patients with COVID-19 had a lower positive rate for CHB[41,42]. One of the postulated

Table 4 Studies showing outcomes and predictors of severity in hepatitis B virus-infected patients with coronavirus disease 2019

Ref.	Study	Patients	Results	Predictors
Yang *et al* [38]	Single centre retrospective study	Patients with HBV infection out of 2899 COVID patients. Resolved hepatitis B (n = 503); HBeAg (-) CHB/infection (n = 44); HBeAg (+) CHB/infection (n = 55); HBV reactivation (n = 6)	HBeAg (+) CHB/infection and HBV reactivation were associated with more abnormal liver function, severe disease, longer ICU stay, and death	Increased ICU admission (HR: 1.86) and mortality (HR: 3.19) in HBeAg (+) CHB/infection
Choe *et al* [40]	Nationwide population-based cohort study	676 chronic HBV infection cases (19160 COVID-19 cases)	Mortality in HBV infected *vs* non-infected patients with COVID-19: 8.2% *vs* 13.5%	No difference in mortality, ICU admission, or organ failure
Wang *et al*[8]	Multicentre retrospective cohort study	109 CHB and 327 non-CHB patients with COVID-19	CHB *vs* non-CHB patients: Severe disease (27.5% *vs* 12.84%) and more dyspnoea (55.05% *vs* 43.12%) and mechanical ventilation requirement (22.49% *vs* 7.95%) in CHB	Increased mortality in CHB patients (OR: 3.748). Predictors of mortality: AST; ALT; ALP; Bilirubin; LDH; Elevated D-dimer. Protective effect: ALB (HR: 0.13); ALB/GLO (HR: 0.123)
Yip *et al*[44]	Retrospective cohort study	Current (353) and past HBV infection (359) out of total 5639 COVID cases	Mortality in current HBV *vs* past HBV *vs* non-HBV infection: 2.3% *vs* 5.8% *vs* 2.2%	Acute liver injury associated with mortality (aHR: 2.45), more than current (aHR: 1.29) or past (aHR: 0.90) HBV infection
Kang *et al* [42]	Nationwide cohort study	7723 COVID-19 cases and 46231 controls	Lower SARS-CoV-2 positivity rate in CHB, after adjusting for comorbidities (aOR: 0.65)	Reduced SARS-CoV-2 positivity (aOR: 0.49) on antivirals
Liu *et al*[39]	Retrospective cohort study	347 COVID-19 patients (21 *vs* 326 with or without chronic HBV infection)	Severe COVID-19 in 30% *vs* 31.4% in the HBV *vs* non-HBV group	Similar SARS-CoV-2 clearance and severe COVID-19

ALB: Albumin; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; COVID: Coronavirus disease; CHB: Chronic hepatitis B; GLO: Globulin; HBV: Hepatitis B virus; LDH: Lactate dehydrogenase.

reasons for this finding is that patients with CHB mount a reduced T cell mediated immune response termed as 'immune exhaustion' which may reduce the extent of cytokine storm seen in patients with COVID-19[41]. The major predictor of poor outcomes was positivity for HBeAg suggestive of active viral replication and ongoing liver injury in addition to liver injury inflicted by SARS-CoV-2[38]. Reactivation of hepatitis B virus (HBV) with anti-IL6 therapy (tocilizumab) was found to be 3.3% in a systematic review[43]. In short, patients with HBeAg positive CHB are more likely to have a poor outcome in terms of hospitalization requirement and mortality[44] and some specific treatment for COVID-19 will lead to reactivation of HBV like anti-IL6 antibody (tocilizumab).

Hepatitis C

Hepatitis C predominantly causes a chronic indolent infection. Various management aspects of hepatitis C have been impacted during the COVID-19 pandemic. The impact of COVID-19 on chronic hepatitis C depends on the extent and severity of underlying CLD as discussed previously. A study by Ronderos *et al*[45] showed an increased mortality among hepatitis C virus (HCV)-infected patients, and increasing age, elevated D-dimer, ferritin, and FIB-4 score were identified as predictors on multivariate analysis. However, more data are required to draw a conclusion regarding the effect of HCV infection on COVID-19, excluding the severity of liver disease.

Autoimmune hepatitis

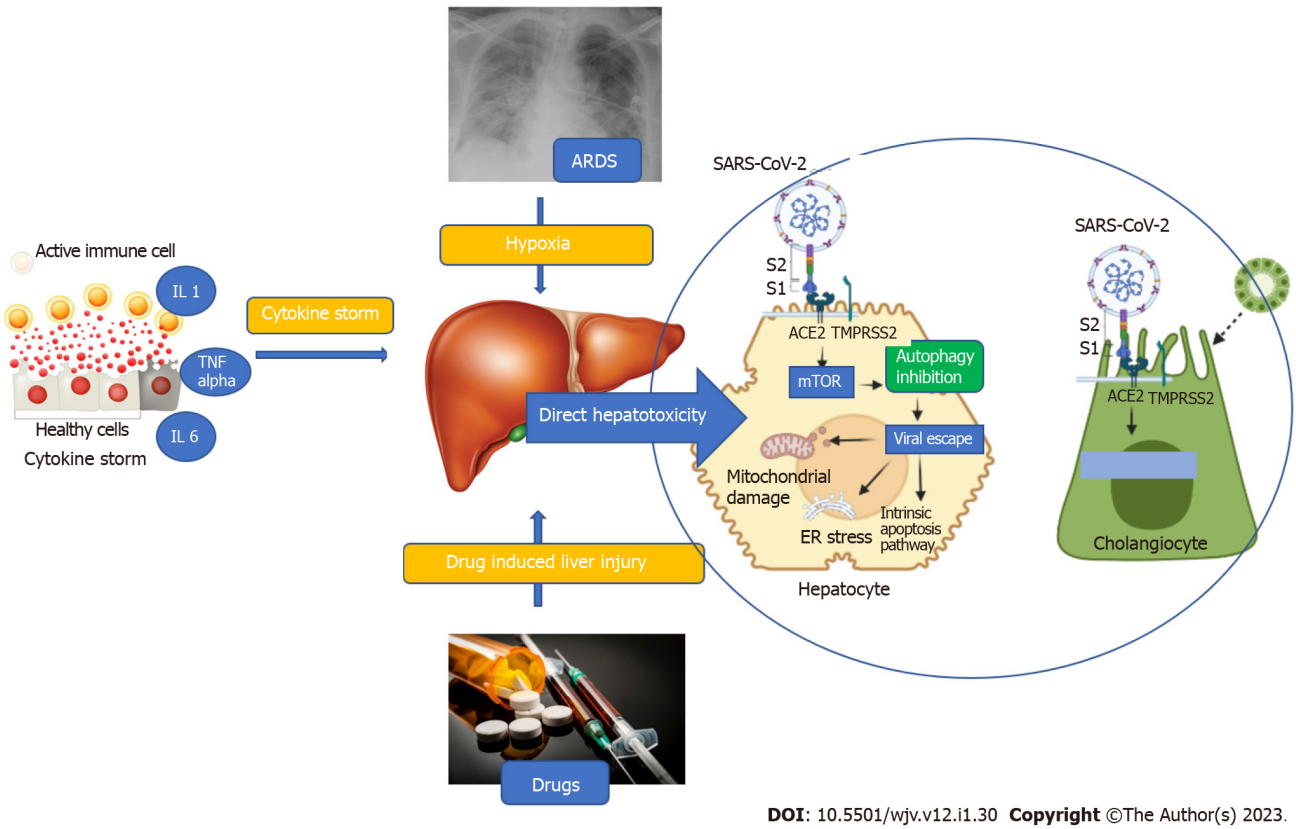
Autoimmune hepatitis (AIH) patients are a vulnerable group due to underlying liver disease, use of immunomodulators, and associated systemic diseases. Different studies have tried to identify the risk factors of severity and outcomes in these patients including those on immunosuppressants. Di Giorgio *et al*[46] demonstrated that the predictors of outcomes were same in AIH as in the general population, including increasing age and presence of comorbidities. Cirrhosis was the most important predictor of mortality among patients with underlying autoimmune liver diseases (OR: 17.46) in a study[47,48]. Among cirrhotics, outcomes worsened with progressive underlying liver dysfunction measured by increasing Child-Pugh-Turcotte (CTP) scores with an OR of mortality increasing from 42 to 69 in Child-Pugh classes B and C, respectively[49] (Table 5).

The effect of immunosuppressive treatment on outcomes in COVID-19 patients has shown some diverging results. A study by Efe *et al*[50] in 254 AIH patients showed that systemic glucocorticoids (aOR: 4.73), thiopurines (aOR: 4.78), mycophenolate mofetil (MMF) (aOR: 3.56), and tacrolimus (aOR: 4.09) were associated with a more severe COVID-19 course. The study showed that outcomes were worse in patients on steroids at a prednisolone equivalent dose of > 5 mg/d. Similarly, another study showed that baseline treatment with steroids, thiopurines, MMF, and tacrolimus were associated with a severe disease course[51]. Therefore, patients with AIH having cirrhosis and stage of cirrhosis reflected

Table 5 Studies evaluating outcomes and predictors in autoimmune hepatitis with coronavirus disease 2019

Ref.	Study	Patients	Results	Predictors of outcomes
Efe *et al* [47]	Multicentre retrospective study from 34 centres in Europe and the Americas	110 AIH patients	Acute liver injury: 37.1%	Predictor of severe COVID-19: cirrhosis (OR: 17.46); Immunosuppression not associated with severe COVID-19 (OR: 0.26)
Di Giorgio *et al*[46]	Phone based survey in tertiary centre	adult AIH patients: AIH (n = 97, 96%); PSC/AIH overlap (n = 2, 2%); PBC/AIH (n = 2, 2%); 4 patients had confirmed COVID	Severe COVID: 1; Death: 1	No difference in risk factors of mortality
Marjot *et al*[49]	Retrospective data from three international registries	70 AIH cases among 932 patients with CLD with COVID-19	No differences between AIH and non-AIH related CLD in Hospitalization (76% *vs* 85%); ICU admission (29% *vs* 23%); Death (23% *vs* 20%)	Factors predicting mortality in AIH: Age (OR: 2.16/10 yr); Child-Pugh class [B (OR: 42.48) and C (OR: 69.30)] cirrhosis
Efe *et al* [50]	Retrospective data from 15 countries	254 AIH patients	Hospitalization: 94 (37%); Death: 18 (7.1%)	Factors associated with COVID-19 severity: Systemic glucocorticoids (aOR: 4.73); Thiopurines (aOR: 4.78); Mycophenolate mofetil (aOR: 3.56); Tacrolimus (aOR: 4.09)

AIH: Autoimmune hepatitis; COVID: Coronavirus disease; CLD: Chronic liver disease; ICU: Intensive care unit; PBC: Primary biliary cholangitis; PSC: Primary sclerosing cholangitis.

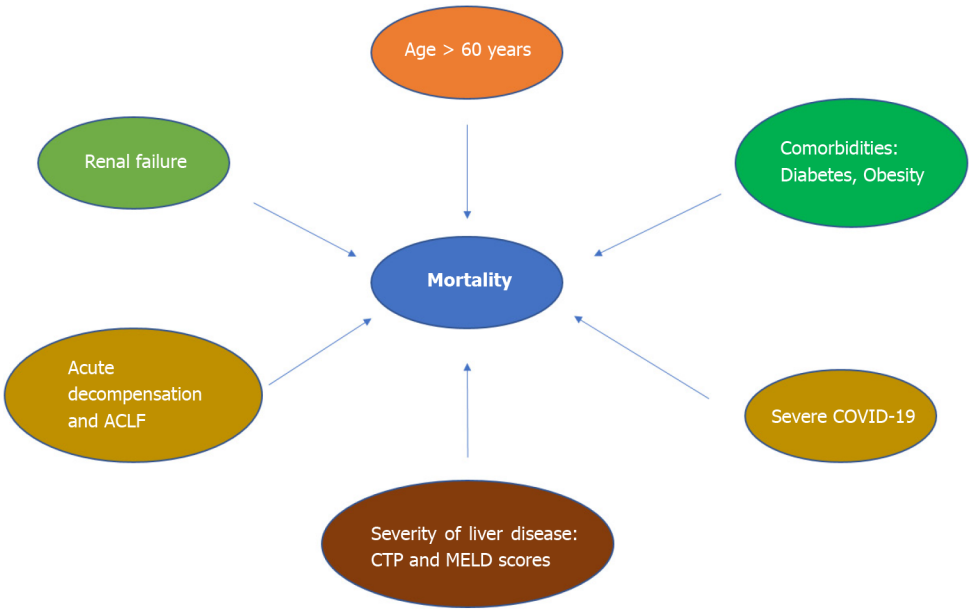


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Figure 1 Pathophysiology of liver involvement in coronavirus disease 2019.

by CTP score are predictors of adverse outcomes. The use of immunosuppressive drugs is also associated with worse outcomes among COVID-19 patients with AIH.

Severity of CLD: One of the most important determinants of clinical outcomes is the presence and severity of underlying cirrhosis as shown in various studies. There was an increasing risk of mortality with increasing CTP score ranging from additional +2% in Child-Pugh A to +20% in Child-Pugh B and +38% in Child-Pugh C. Similarly, another study showed that Child-Pugh score was associated with mortality[52]. CTP score of more than 9 was associated with a high mortality (HR: 19) in another study



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Figure 2 Predictors of mortality in pre-existing chronic liver disease with coronavirus disease 2019. ACLF: Acute-on-chronic liver failure; CTP: Child-Turcotte-Pugh score; COVID-19: Coronavirus disease 2019; MELD: Model for end stage liver disease.

[37]. Mortality in cirrhotics increased with worsening chronic liver failure consortium score (HR: 1.42), which is an indicator of hepatic and extrahepatic organ failures[53]. Model for end stage liver disease (MELD) score more than 25 is associated with a two-fold increase in mortality as demonstrated by univariate analysis in a study from India[24]. Similarly, a study from Italy showed that MELD score more than 15 was associated with a higher mortality (HR: 5.18) at 30 d[53]. Various factors associated with adverse outcomes in cirrhosis are summarized in Table 1. Therefore, the currently available evidence suggests that increasing severity of underlying CLD is associated with poor outcomes including mortality.

Degree of liver fibrosis: A study from China showed that higher FIB-4 score, which is a marker of liver fibrosis, was associated with a more severe COVID-19 disease[36] with greater requirement of high flow oxygen, prolonged hospitalization, and even death[54]. They postulated that FIB-4 score could be a prognostic marker of disease outcomes but more data is required to increase external validity. Similar findings were seen in a meta-analysis which showed that elevated FIB-4 score was associated with severe COVID-19 and mortality[54]. Hence, the degree of liver fibrosis is an important determinant of disease outcome with higher degree of fibrosis being negatively associated with outcomes[55].

Comorbidities: Another predictor of mortality was the presence of comorbidities, with the most common being diabetes, obesity, dyslipidemia, and hypertension[16-18,37]. This may be due to a more severe COVID-19 infection seen in this subgroup of patients irrespective of the presence of cirrhosis. A prospective study showed that a BMI of more than 30 was associated with mortality[17]. Another study showed that diabetes and hypertension were predictors of mortality[18]. A study from Asia showed that diabetes was associated with severe liver injury without cirrhosis (OR: 2.1), as was obesity in cirrhotics (OR: 8.1)[37]. Therefore, the presence of comorbidities increases the severity of liver disease and has unfavorable outcomes.

Severity of COVID-19: Some studies showed that respiratory failure was the main cause of mortality among cirrhotic COVID-19 patients. Outcomes were poor for patients with higher CURB-65 (confusion, uremia, respiratory rate, blood pressure, age > 65) scores, substantiating the fact that respiratory failure was associated with mortality[44]. In this study, they observed that CURB-65 score was associated with a 5-fold increased risk of mortality[52]. Severe COVID-19 with respiratory failure was a significant predictor of mortality (HR: 2.5) in patients with chronic liver disease in another study[24]. Henceforth, more severe COVID-19 is associated with an increased risk of liver injury and mortality.

Biomarkers: A recent study evaluated the role of inflammatory biomarkers in risk-stratifying the patients with regard to liver injury and mortality in 221 COVID-19 patients[56]. They included CRP, IL6, D-dimer, and blood lymphocyte counts as inflammatory biomarkers, which were all significantly elevated in the patients who subsequently expired as compared to survivors. They found that patients who showed rising aspartate transaminase and alkaline phosphatase over time, as markers of liver

injury, had a higher mortality. These correlations attenuated with age. Thus, inflammatory biomarkers may serve as predictors of poor outcomes, but more studies are required for identification of biomarkers and their validation.

Miscellaneous factors: Some other factors affecting the severity and outcomes of COVID-19 have also been seen in some studies. As discussed previously, obesity and physical inactivity have been associated with worse outcomes. Recently, it has been shown that obesity, physical inactivity, and diet rich in simple sugars predisposes to chronic low-grade inflammation in the mucosal barrier along with microbial dysbiosis. This state of chronic inflammation has been shown to be associated with worse clinical outcomes in COVID-19 patients[57]. Similarly, obese individuals with excessive visceral fat have excessive proinflammatory adipokines that have been postulated to be associated with poor outcomes [57]. Extrapolating the role of inflammation, patients with CLD have low level endotoxemia with increased gut permeability which may be associated with unfavorable outcomes; however, concrete evidence for the same is lacking[58]. A summary of risk factors associated with poor outcomes is shown in Table 6.

PREDICTORS RELATED TO OUTCOMES OF UNDERLYING CHRONIC LIVER DISEASE

Acute decompensation and acute-on-chronic liver failure

In patients with CLD, acute decompensation and acute-on-chronic liver failure (ACLF) usually develop due to a precipitating factor, with infections being the most common such factor. COVID-19 may act as a trigger for such decompensation. Marjot *et al*[22] observed in their study that the major predictor of decompensation was Child-Pugh class, with a rate of decompensation of 30%, 56%, and 64% observed in Child-Pugh A, B, and C patients, respectively. Hepatic decompensation at baseline was associated with an increased mortality (HR: 2.91) in another study[18]. Acute decompensation developed in 9% and ACLF in 11.6% among 43 cirrhotic patients, and CTP score was the major predictor of mortality, with a CTP score of 9 or more at presentation associated with high mortality (HR, 19.2)[37]. Another study showed that acute decompensation developed in 62.9% and ACLF in 29% with a mortality as high as 72% among ACLF patients, with major predictors of mortality being MELD score, leukocytosis, elevated creatinine, and COVID-19 severity on multivariate analysis[24]. The mortality in grades 1, 2, and 3 ACLF patients was 56.3%, 50%, and 93.3%, respectively (P = 0.001) in the same study. In a prospective study of 96 cirrhotic patients, 61.4% developed acute decompensation and ACLF in 55% according to CLIF-C criteria. The major predictors of mortality were CLIF-C organ failure score (AUROC: 0.85) and MELD Na score (AUROC: 0.70)[17].

These observations suggest that SARS-CoV-2 infection may be a triggering factor for decompensation and subsequent ACLF in cirrhotic patients by triggering a pro-inflammatory cascade as discussed earlier. Possible factors that can be postulated could be a proinflammatory milieu, multi-organ dysfunction due to severe COVID-19, direct hepatotoxicity to a compromised liver, or sepsis. In summary, COVID-19 patients with underlying CLD are more prone to develop acute decompensation and may progress to ACLF. The major predictors of these outcomes are the baseline severity of liver disease reflected by CTP and MELD score, and the severity of hepatic and extra-hepatic organ failure as indicated by CLIF-C scores.

Upper gastrointestinal bleeding

The data on the rate and risk factors of variceal bleed in patients with COVID-19 positive patients with underlying CLD is scarce. Upper gastrointestinal (UGI) bleeding developed in 24/1342 (1.8%) of all patients admitted with COVID-19[24]. Most of bleeding episodes (88%) were variceal bleeding in patients with cirrhosis with no rebleeding or death at 5 d with medical management alone[24]. The same group also observed that the initial control of UGI bleeding was achieved in all patients with no one requiring an emergency endoscopy. Thus, emphasizing the utility of conservative management of variceal bleeding with endoscopic therapy is only needed on a case-to-case basis[25].

Another study from Hong Kong showed that although peptic ulcer bleeding was the most common cause of UGI bleeding both before (66.0%) and during (66.1%) the COVID-19 pandemic, there was a significant increase in the proportion of patients with UGI bleeding with variceal bleeding after COVID-19 (5.3% *vs* 10.5%, P < 0.01)[51]. Patients had significantly lower hemoglobin (7.5 *vs* baseline 8.3 g/dL) and higher requirement for blood transfusion (64.5% *vs* baseline 50.4%) but had similar rates of all-cause mortality (6.9% *vs* 7.1%) and rebleeding (6.7% *vs* 5.1%)[59]. There was no significant difference in the timing of endoscopy after admission or the percentage of patients requiring endoscopic hemostasis (77.3% *vs* 76.3%) before and during the COVID-19 pandemic[59]. Thus, patients with variceal bleeding in the COVID-19 pandemic have similar management principles as the pre-COVID-19 era.

HCC

The major impact of the COVID-19 pandemic on patients with HCC was multifactorial. A decline of

Table 6 Risk factors associated with adverse outcomes in coronavirus disease 2019 affected patients with chronic liver disease

Demographics	Etiology	Clinical parameters	Underlying disease severity	Biochemical parameters
Age > 60 yr; Hispanic and black ethnicity; Diabetes mellitus; Hypertension; Obesity	Alcohol; HBeAg positivity among CHB; AIH on immunosuppressants	Respiratory failure: CURB-65 score; Decompensation at baseline; ACLF at presentation	CTP score; MELD score; FIB-4 index	Elevated creatinine; Leucocytosis; AST levels; ALT levels; CRP

ACLF: Acute-on-chronic liver failure; AIH: Autoimmune hepatitis; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CRP: C-reactive protein; CTP: Child-Turcotte-Pugh score; CURB-65: Confusion, Uremia, Respiratory rate, BP, age > 65 years, FIB-4: Fibrosis 4 score; MELD: Model for end stage liver disease.

26.7% in new HCC cases was reported during the pandemic compared to the pre-pandemic era[60]. Advanced BCLC stage and higher tumor burden at diagnosis were due to resource limitation and lack of physical appointments, and were associated with a higher incidence of spontaneous tumoral hemorrhage[61]. Delayed treatment initiation longer than 1 month (21.5% *vs* 9.5%; $P < 0.001$) due to re-allocation of services for the pandemic was reported[62]. Muñoz-Martínez *et al*[63] reported an increase in mortality rate proportional to advanced BCLC stage. Thus, the impact of COVID-19 on patients with HCC is predominantly due to delayed diagnosis, delayed presentation, delay in initiating treatment, and availability of imaging and locoregional or transplant facilities.

COVID-19 waves and impact on liver disease

Some studies identified the impact of different waves of the pandemic on liver disease. The waves of COVID-19 occurred due to mutations and spread of newer variants of the virus that evaded the immune response. The second wave was predominantly caused by delta variant[64]. Nawghare *et al*[65] showed that the second wave had more number of acute decompensations and the factors predicting outcomes were renal dysfunction and elevated D-dimer. Elhence *et al*[24] compared outcomes in the first wave to those in the second wave and reported that although the disease severity was more during the second wave but the mortality rate and duration of hospital stay were similar with no significant differences.

CONCLUSION

COVID-19 has a major impact on patients with pre-existing CLD in the form of severe COVID infections and worsening of underlying hepatic disease. The predictors of poor outcomes of COVID-19 patients with underlying CLD are multiple and have been different in numerous studies across the globe, with the most important predictor being presence of cirrhosis with outcomes progressively deteriorating with increasing severity of underlying liver dysfunction estimated by CTP and MELD scores. These are the subgroups of patients who are more prone to risk of decompensation, further decompensation, and ACLF.

The predictors may be related to demographic factors, with increasing age and black and Hispanic ethnicity being associated with poor outcomes. Another major predictor of the severity of COVID-19 is cytokine storm, which may even lead to multiorgan failure, with the liver being one of the organs involved. Other predictors include the presence of comorbidities, whose prevalence is estimated to be around 30%-50% in various studies, and these have been associated with poor outcomes even in the absence of underlying liver disease. Major comorbidities found in studies that are negatively associated with outcomes include diabetes mellitus, hypertension, and obesity. The COVID-19 pandemic also adversely affected routine services for patients with hepatitis B, hepatitis C, and HCC, which will have long-term impacts in the form of increased disease burden, delayed implementation of eradication programs, and poor outcomes in the times yet to come.

FOOTNOTES

Author contributions: Walia D, Saraya A, and Gunjan D contributed equally to this work; Walia D drafted and edited the manuscript; Saraya A and Gunjan D drafted and critically revised the manuscript; all authors have read and approved the final manuscript

Conflict-of-interest statement: All the authors have none to declare for this article.

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MINIREVIEWS

Commentary on COVID-19-induced liver injury in various age and risk groups

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Abstract

Towards the end of 2019, a new type of coronavirus, severe acute respiratory syndrome, emerged in the city of Wuhan in China's Hubei Province. The first occurrence was described as a case of pneumonia. Coronavirus disease 2019 (COVID-19) can progress primarily with symptoms varying from a mild upper respiratory tract infection to severe pneumonia, acute respiratory distress syndrome, and death. Determining the mechanisms of action of this virus, which can affect all systems including gastrointestinal, is vital for predicting the progression of the disease and managing its treatment. It is important to demonstrate the mechanisms of action of COVID-19 in patients without a previously known chronic or systemic disease. Although there is still no specific treatment for the virus, various algorithms have been created. As a result of the applied algorithms, the response to the treatment was satisfactory in some patients, while unexpected side effects occurred in some patients. It helps to clarify whether the unwanted effects that occur are due to the effect of the disease or the side effects of the drugs used in the treatment. There is currently increasing interest in COVID-19 interaction with liver tissue. Therefore, we would like to discuss the details of liver injury/dysfunction in the current literature.

Key Words: COVID-19; Liver injury; SARS-CoV-2

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Core Tip: Coronavirus disease 2019 (COVID-19) can progress primarily with symptoms ranging from a mild upper respiratory tract infection to severe pneumonia, hepatic injury, and even death. There is currently increasing interest in COVID-19 interaction with liver tissue. We would like to discuss the details of liver injury/dysfunction in COVID-19 in the current literature.

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INTRODUCTION

Hepatotropic viruses replicate in their main target liver, which can be involved in these viruses' infections. The host is mostly afflicted as a consequence of the immune response to viruses like hepatitis A, hepatitis B, hepatitis C, and hepatitis E viruses which can be a known reason for hepatitis and liver damage[1]. In non-hepatotropic viral infections like severe acute respiratory syndrome (SARS), Epstein-Barr virus infection, *etc.*, it is known that the liver is mainly affected as a result of immune infiltrates and reactions that occur as a result of the virus-induced immune system response. The result of this effect can range from mildly irregular liver biochemistry to fulminant hepatic failure. The liver is also affected by infections such as adenovirus, cytomegalovirus, and other opportunistic viruses in people with immunocompromised and other immune system disorders[2].

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is identified to result in severe acute respiratory syndrome through its host cell receptor, angiotensin-converting enzyme 2 (ACE-2). As a part of multi-organ involvement, cardiac, kidney, and liver injury can be seen[3]. The fact that coronavirus disease 2019 (COVID-19) is more important than diseases caused by many other viruses and needs to be investigated is its high mortality. In a meta-analysis of 3772 patients acquired from 326 studies examining SARS-CoV-2 and liver damage, it was demonstrated that there is a link between liver dysfunction and fatality[4]. Interestingly, besides the respiratory system, a significant proportion of SARS and COVID-19 patients showed signs of liver damage of varying degrees, the mechanism and effect of which have yet to be determined (Table 1).

PREVALENCE OF LIVER INJURY IN COVID-19

The prevalence of liver damage in COVID-19 patients differed from 16% to 29%. A meta-analysis showed that the rate of liver dysfunction among COVID-19 cases was 27.4%[5,6]. Fu *et al*[7] collected data from 355 patients in China and demonstrated that 39.6% of COVID-19 cases were afflicted with cholestasis, 51.9% with hypoproteinemia, and 39.0% with hepatocellular injury at presentation.

SUPPOSED MECHANISMS OF LIVER INJURY

The etiology of acute liver injury in COVID-19 patients remains unclear but is likely to be multifactorial (Table 1). It is supposed that it may be due to the direct invasion of hepatocytes by viruses, immune-mediated damage, the toxicity of drugs utilized in the treatment, hypoxia, ischemia, endothelial dysfunction, microthrombi formation, systemic inflammatory response syndrome, sepsis, or exacerbation of underlying liver disease[8].

ACE-2 receptors can be demonstrated in hepatic cholangiocytes and hepatocytes. These receptors make the gastrointestinal system a target for SARS-CoV-2 infection, which can vigorously infect and reproduce. The strong affinity of the SARS-CoV-2, particularly to cholangiocytes, results from a high binding rate to the ACE-2 receptor, which suggests that it is related to impaired hepatic function[9,10]. In a study, cell type-specific expression of ACE-2 was investigated in healthy liver tissues. The researchers showed that the virus can bind directly to ACE-2-positive cholangiocytes, but not hepatocytes. This finding suggests that liver abnormalities of SARS and COVID-19 patients are due to cholangiocyte dysfunction, not hepatocyte damage. What is confusing at this point is that in many studies conducted in China, elevation in aspartate aminotransferase (AST) instead of γ-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP) indicates cholangiocyte and bile duct damage [11]. But it should be in mind that this occurs only infrequently on hepatocytes, nevertheless it may be upregulated on hepatocytes during periods of physiologic stress[12]. Myalgia or myositis are also common symptoms in COVID-19 patients. Therefore, it may be necessary to examine the elevations in lactate dehydrogenase and creatinine kinase in COVID-19. The AST elevations observed in this circumstance can be ascribed to some degree to muscle injury[13].

In addition, it is known that patients with COVID-19 have severe hypoxia, and this hypoxemia significantly affects all organs, including the liver. As a part of SARS-CoV-2 infection in severe acute respiratory syndrome, capability to trigger severe hypoxia that was recalcitrant to the management of high inspired fractions of oxygen and high mean airway pressures was observed. Hypoxic hepatic damage is noticeable by alanine aminotransferase (ALT)/AST elevations owing to oxygen imbalance.

Table 1 Events in the liver from a broad perspective of severe acute respiratory syndrome coronavirus 2 infection

Effect	Mechanism	Result	Outcomes and morphological changes
SARS-CoV-2 virus	Genomic translations and replication	More viruses in circulation	Inflammation features. Usually, the biliary intrahepatic tree and bile duct did not show any significant histological alteration. Actin smooth muscle antibodies existed in pericytes which were in portal vein walls and adventitial areas
Viral proteins of SARS-CoV-2	SIRS -> stimulate cytokine storm	Increased TNF-α, IL-6, IL-1β, IL-2, IL-8, CCL2, CCL3, CCL5, CXCL10 levels. Decreased ($CD4^+$) T cell and NK cell counts	Increase in the number of portal vein branches associated with lumen massive dilatation and focal periportal abnormal vessels. Portal vein endotheliitis (fragmented smooth muscle layer). Scattered portal and lobular lymphocytes. Extremely activated Kupffer cells with large cytoplasm containing necrotic debris
Hypoxia	Hypoxic ischemic injury of all organs and also liver	Decreased SpO2 levels, mitochondrial dysfunction, and hypoxic hepatocytes express higher ACE-2 levels	Partial or complete luminal thrombosis of the portal and sinusoidal vessels, focal portal vein parietal fibrosis, enlarged and fibrotic vessels. A diffuse network of sinusoids decorated by CD34 suggests a disturbed circulation of blood within the liver
Drugs (antivirals, immune stimulants)	Liver damage	Increase in ALT, AST, LDH, CRP, D-dimer, ferritin, and bilirubin levels and a decrease in albumin levels	Portal fibrosis, lobular and mild portal inflammation
Drugs and viral proteins	The cytopathic effect, oxidative imbalance	Apoptosis and steatosis	Small and/or large droplets of steatosis in hepatocytes

ACE-2: Angiotensin-converting enzyme 2; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; LDH: Lactate dehydrogenase; CRP: C-reactive protein; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; SIRS: Systemic inflammatory response syndrome; TNF-α: Tumour necrosis factor-alpha; IL: Interleukin.

This shows that severe hypoxia may be one of the pathophysiological elements of hepatic injury in COVID-19[13,14].

Therapeutic agents, *e.g.*, hydroxychloroquine, immune modulators (tocilizumab, steroids, and anakinra), anti-retroviral drugs (remdesivir, favipiravir, and lopinavir), antibiotics (azithromycin and ceftriaxone), and antipyretics (paracetamol and ibuprofen), which are utilized in the management of COVID-19, also have hepatotoxic effects. Patients are recommended to have regular follow-ups who have management with single and/or combined use of these potential hepatotoxic drugs for possible hepatic injury[8,13].

The existence of viral particles in the feces of infected cases indicates that the gastrointestinal system is affected by SARS-CoV-2. This is another reflection that demonstrates the possible direct impact of SARS-CoV-2 on liver tissue, provided the close association between the bowel and the liver. The exact mechanisms of this suggested direct injury route are yet to be explained[15]. Even the gut microbiome is important at this point since ACE-2 is also expressed in the luminal intestinal epithelium. With the attachment of SARS-CoV-2 to the ACE-2 receptor, intestinal permeability and inflammation increase. All these events increase the risk of bacterial translocation, which leads to dysregulation of the gut microbiome and, *via* this pathway, to Gram-negative sepsis, through portal circulation[16]. This translocation is also partially responsible for liver damage in COVID-19 patients[17].

PATHOGENESIS

SARS-CoV-2 is a new positive-strand RNA virus from the beta coronavirus family and has a glycolipid envelope. The virus connects to the host's ACE-2 receptor to start an infection. The viral access and reproduction process begins. ACE-2 is existing in cardiomyocytes and most endothelial cells except for those lining the liver sinusoids, lungs, bile ducts, bowels, and kidneys[18]. The spike protein (S protein) is located on the SARS-CoV-2 surface that will attach to ACE-2. After binding to the cell membrane, the virus is detained when the viral envelope fuses with the host membrane. Moreover, the type 2 transmembrane serine protease, which is present in host target cells (predominantly alveolar epithelial type II cells), stimulates viral uptake. The viral genome accesses the cytoplasm and is converted to produce new virions. After the virus enters the cell by fusion with the host membrane, an antiviral immune response begins with the viral nucleocapsid proteins remaining on the cell surface. These viral nucleocapsid proteins are recognized by antigen-presenting cells. Viral antigens are passed to cytotoxic ($CD8^+$) and regulatory ($CD4^+$) T lymphocytes by major histocompatibility complexes, also known as human leukocyte antigens[19].

Viral-specific $CD8^+$ T cells stimulated as a response to viruses affecting organs other than the liver are thought to be involved in the manifestation of T cell-mediated hepatitis lacking viral antigens in the

liver. Even with the extrahepatic influence of viruses, liver damage can be seen when no virus is detected in the liver. Because most strains of these viruses infect only the epithelial cells of the airway and therefore viral antigens should not be existing in the liver. This phenomenon was detected by the effect of the influenza virus and defined as collateral damage[20].

Previous studies on viral infections other than SARS-CoV-2 are therefore important and show that the liver is involved in diseases not only by antigen-specific T-cell response activation but also by some clinical hepatic inflammation syndromes that are not easily explained. It is expected that lymphocytes are caught in the liver sinusoids and cause occlusion, reducing blood flow, and infiltrates consisting of inactivated lymphocytes are expected to increase liver damage. All of these and more may cause more liver damage with the effect of autoimmunity. Exacerbated inflammation in response to SARS-CoV-2 is also a cause of immune damage. The immense discharge of cytokines by the immune system in reaction to the viral infection can cause a cytokine storm and symptoms of sepsis that are the reasons for fatality in 28% of mortal COVID-19 patients[21].

OTHER ETIOPATHOGENETIC FACTORS IN LIVER DAMAGE INDUCED BY SARS-COV-2

Preexisting liver diseases

The relationship between chronic or dormant liver diseases and COVID-19 has been investigated in many studies. In the frequency of COVID-19, a higher number has not been determined in patients with previous liver disease, unlike the general population. Two to five percent of COVID-19 cases had known liver disease before contracting COVID-19. However, there is an opinion that there may be an increase in severe COVID-19 and death in chronic liver patients[5]. As liver damage increases, patients begin to worsen in their clinical condition and prognosis. Established hepatic diseases may have adversarial effects on COVID-19 prognosis, including severity, fatality, and need for mechanical ventilation[22].

Liver transplantation

After the liver transplantation in approximately 700 children with chronic liver disease, only three had SARS-CoV-2 infection and no lung or other system disease such as pneumonia was observed in these patients[23]. Also, in a different study that included data on 151 Liver transplant adults, six recipients fell ill with COVID-19, while three long-term liver transplant recipients died. Data from liver transplant recipients continue to be organized in the form of case reports[24]. It should be kept in mind that patients with liver transplantation receive immunosuppressive treatments and these treatments may initiate cytokine storms or increase viral spread with an asymptomatic course in mild COVID-19 infections[25].

Cholestatic liver disease

Considering the cells and organs where ACE-2 receptors are located, it is expected to be affected in cholestatic liver disease. The fatality rate was higher in patients with cholestasis[22].

Cirrhosis

The incidence of COVID-19 varies widely in patients with cirrhosis. In a study that analyses cirrhosis patients in China, it was reported that 5 of 16 cirrhosis patients died[26]. The reason can be cirrhosis-associated immune dysfunction. They are more likely to have poor outcomes from ARDS.

Other liver diseases

All liver diseases have interactions with COVID-19 resulting from their specific disorder[26]. Chronic low-grade inflammation known to be associated with metabolic dysfunction-associated fatty liver disease may worsen COVID-19 outcomes[22,27,28].

EFFECTS OF VARIOUS AGE AND RISK GROUPS ON LIVER INJURY

When the literature is examined, the striking point is that SARS-CoV-2 does not occur with the same clinical features in patients. Since the severity of the disease is also related to the liver, liver involvement is not the same in every patient. Liver injury varies in patients having previously known blood disease, susceptibility to thrombosis for portal/hepatic thrombosis or immunosuppression, *etc.*[29]. Risk factors such as age, gender, previous diseases, chronic or acute health status, various medications, coronary artery diseases, metabolic diseases, serologies for other viruses related to hepatitis, and other etiologies affect the severity of the disease[3,8].

Elevated liver enzymes were more frequent in males with severe COVID-19 than in females[30]. Also, male gender, older age, and lymphopenia were three important independent risk elements forecasting hepatic dysfunction among COVID-19 cases[7]. Additionally, a clinical study showed that pulmonary failure was related to poor prognostic indicators of hepatic failure[31].

When the pediatric group is examined, only scarcely data is in the pediatric literature. We believe that the reason for this may be a milder course or asymptomatic transmission of SARS-CoV-2 infection in children[32]. In the data of a study that examined SARS-CoV-2 infection by dividing it into two groups, the data of children who had COVID-19, the first group, and that of multi-system inflammatory syndrome in children and adolescents (MIS-C), the second group, were shared. Elevated ALT was found in 36% of the 291 patients, with 31% having COVID-19, and 51% having MIS-C. High levels of ALT in COVID-19 were accompanied by obesity, immune-compromised status, and chronic hepatic disease. Children with elevated ALT and MIS-C were more often boys. Children with MIS-C had a 2.3-fold augmented risk of high ALT compared to COVID-19. No relationship was detected between elevated ALT and fatality[29].

EFFECTS OF DRUGS ON COVID-19-INDUCED LIVER INJURY

With the onset of the pandemic, various guidelines have been published for the treatment algorithm for COVID-19. Some drugs have been removed from the list due to their side effects and benefits. Currently, no specific drug for the SARS-CoV-2 virus has been discovered, and studies on this subject are continuing. The effects of the commonly used medications on the liver are examined.

Remdesivir

Remdesivir is a nucleotide analog prodrug with antiviral activity against SARS-CoV-2. Recently, the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) endorsed remdesivir for the management of cases admitted with severe COVID-19. The most common adverse drug reaction reported in hospitalized patients treated with remdesivir with a diagnosis of severe COVID-19 was elevations in liver enzymes. There is limited data on the number of patients exposed to remdesivir in clinical studies reporting severe hepatotoxicity or drug-induced liver injury[33].

Adverse effects reported in at least 5% of all patients in remdesivir trials were decreased glomerular filtration rate, a decline in hemoglobin level, decreased lymphocyte count, pyrexia, increase in blood glucose and creatinine level, transaminase elevations, *etc.*, whose rates were generally similar between remdesivir and placebo[34]. In general, when the adverse events in the remdesivir group and the placebo group were compared, adversarial events were detected in 102 (66%) of 155 remdesivir users and 50 (64%) of 78 placebo users. However, 18 (12%) patients in the remdesivir group and 4 (5%) patients in the placebo group had to be discontinued early due to side effects (including gastrointestinal symptoms, aminotransferase or bilirubin elevations, and worsening cardiopulmonary status), which is more frequent with remdesivir than with placebo. However, when we examined the liver enzymes in cases where treatment was required to be terminated, it was observed that there was an indication in only three (2%) patients in the remdesivir group due to the increase in aspartate aminotransferase[35]. In a more comprehensive study describing the drug-induced liver injury and liver disorders caused by remdesivir, the data were different. Among 387 events with remdesivir listed in VigiBase, 130 hepatic adverse events (34%) were described; they were the most frequent adverse drug reactions. One hundred and fourteen cases had elevated liver transaminases. A more pronounced correlation of the incidence of hepatic failure has been reported with the use of remdesivir compared with hydroxychloroquine, lopinavir/ritonavir, or tocilizumab (odds ratio, 1.94; 95% confidence interval, 1.54-2.45)[36].

Baricitinib and JAK (Janus kinase) inhibitors

Tofacitinib and baricitinib are immunomodulators that are thought to have potent antiviral effects through interference with viral entry. There is not enough data on the side effects on the liver in clinical trials[37].

IL-6 pathway inhibitors (e.g., tocilizumab)

Serious adverse events in the trials were not greater with IL-6 pathway inhibitors than with comparators. It has been discussed in some studies that these inhibitors may be associated with an increased risk of secondary infections[38]. Tocilizumab and baricitinib which are used widely in COVID-19 may also cause HBV reactivation[37].

Glucocorticosteroids

Glucocorticosteroids may result in hepatic steatosis (fatty liver) that can infrequently cause systemic fat embolism or cirrhosis as an adverse effect[39].

Non-steroidal anti-inflammatory drugs

The use of paracetamol was considered relatively safe after the especially beginning of the COVID-19 epidemic and the effect of ibuprofen on ACE receptors was revealed. Therefore, patients suffering from COVID-19 frequently consumed paracetamol for its antipyretic and analgesic effects[40]. It is known that the effects of paracetamol are generally dose-dependent. However, hepatotoxicity risk can be seen

at levels much lower than the expected dose, even at therapeutic doses. At this point, genetic characteristics and metabolic differences may be due to immune-mediated mechanisms[41].

Non-steroidal anti-inflammatory drugs (NSAIDs) impede cyclooxygenase (COX)-1 and COX-2. Significant and well-known common side effects are on the gastrointestinal and renal systems. There are cases of liver toxicity reported and frequently encountered in the literature[42]. Currently, there is no strong evidence about the safety of the use of NSAIDs in COVID-19 patients[43].

HISTOLOGICAL FINDINGS OF LIVER INJURY INDUCED BY SARS-COV-2

Hepatic histology in cases with COVID-19 is nonspecific, comprising moderate microvesicular steatosis with mild, mixed lobular and portal activity and focal necrosis. In a series of 48 autopsies, pathologic hepatic outcomes consisted of focal portal and lobular lymphocytic infiltrates and changes indicative of hepatic vascular participation[44]. In another study conducted with the liver samples of 40 patients who died due to COVID-19, hepatic involvement was observed in all of the patients. Macrovesicular steatosis was the most frequent (30 cases, 75%), followed by mild lobular necroinflammation and portal inflammation (20 patients each, 50%). Vascular pathology, including sinusoidal microthrombi, was rare and observed in six (15%) cases. PCR using hepatic tissue samples was positive in 11 of 20 cases tested, but quantifying viral load in the liver is lacking[45].

DIFFERENT LABORATORY FINDINGS INDICATIVE OF LIVER INJURY INDUCED BY SARS-COV-2

An increase in liver transaminases has been detected in approximately two-thirds of patients with severe COVID-19. The analysis demonstrated that the more severe the coronavirus infection, the greater the levels of ALT, AST, total bilirubin, ALP, and GGT, and the lesser the level of albumin[46]. Mean levels of AST and ALT over 400 U/L have been reported[47]. Low albumin has been associated with severe COVID-19. Nevertheless, it is uncertain if hypoalbuminemia is a risk element for severe COVID-19 or if hypoalbuminemia is a consequence of severe COVID-19[7,48]. Although they are rare, cases progressing from liver damage to ischemia have been reported[49]. Liver functional indexes of two-thirds of COVID-19 cases stay abnormal 14 d after discharge[7].

BRIEF SUMMARY OF OUR OPINION

COVID-19 is still a multisystemic disease with many unknowns. It is known today that it affects the liver, albeit indirectly, as it has on all systems. Due to its mortal course, the disease has been tried to be treated with rapidly created emergency treatments and algorithms. However, the effectiveness of these treatments, which are performed without knowing the effects of SARS-CoV-2 on the organs, is controversial. It is an already known fact that more studies are needed on the virus and the pathogenesis of COVID-19. However, the side effects of drugs should also be analyzed in detail. The treatment and algorithms of liver failure, especially seen in the severe patient group, are confusing. Things to do in liver involvement due to virus and liver effects that can be seen due to drug side effects may be completely different. While it is necessary to ensure that the liver recovers from the cytopathic effects of the virus with the least damage and continues to fight the disease, it should also be noted that liver failure can be triggered by the drugs applied for this. In these two different situations, different procedures need to be implemented. At this point, we think that histopathological data can be used as we have mentioned in detail in our article. We would like to draw attention to the fact that most of the histopathology data were made postmortem in the studies. We can suggest that liver biopsy should be performed in patients with appropriate clinical status before the treatment procedures are discontinued or changed, and histopathological and immunological examinations should be on the agenda. Although liver biopsy is an invasive and costly procedure, we think that the data obtained may be useful in explaining the liver pathologies of the patients, as well as in providing an idea about the side effects of drugs and in the follow-up of the patients.

CONCLUSION

Liver diseases in COVID-19 have been studied in different groups, from mild to severe and chronic. In light of these studies, our general opinion is that SARS-CoV-2 infection will be more fatal in the case of liver disease. We think that both the direct injury to the liver and other etiological factors including the drugs used have an effect. Exploring liver injury related to COVID-19 has an important role in the

estimation of fatality and might be used for the creation of prognostic tools to recognize cases with possible worse consequences.

FOOTNOTES

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SYSTEMATIC REVIEWS

COVID-19-related liver injury: Focus on genetic and drug-induced perspectives

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Abstract

BACKGROUND

Empirical use of potentially hepatotoxic drugs in the management of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is considered as one of the major etiopathogenetic factors for liver injury. Recent evidence has shown that an underlying genetic factor may also occur. Hence, it is important to understand the host genetics and iatrogenic-based mechanisms for liver dysfunction to make timely remedial measures.

AIM

To investigate drug-induced and genetic perspectives for the development of coronavirus disease 2019 (COVID-19)-related liver injury.

METHODS

Reference Citation Analysis, PubMed, Google Scholar and China National Knowledge Infrastructure were searched by employing the relevant MeSH keywords and pertaining data of the duration, site and type of study, sample size with any subgroups and drug-induced liver injury outcome. Genetic aspects were extracted from the most current pertinent publications.

RESULTS

In all studies, the hepatic specific aminotransferase and other biochemical indices were more than their prescribed upper normal limit in COVID-19 patients and were found to be significantly related with the gravity of disease, hospital stay, number of COVID-19 treatment drugs and worse clinical outcomes. In addition, membrane bound O-acyltransferase domain containing 7 rs641738, rs11385942 G>GA at chromosome 3 gene cluster and rs657152 C>A at ABO blood locus was significantly associated with severity of livery injury in admitted SARS-CoV-2 patients.

CONCLUSION

Hepatic dysfunction in SARS-CoV-2 infection could be the result of individual

drugs or due to drug-drug interactions and may be in a subset of patients with a genetic propensity. Thus, serial estimation of hepatic indices in hospitalized SARS-CoV-2 patients should be done to make timely corrective actions for iatrogenic causes to avoid clinical deterioration. Additional molecular and translational research is warranted in this regard.

Key Words: SARS-CoV-2; Liver injury; Genetic prospective; Drug-induced liver injury; Prognosis; COVID-19

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Core Tip: Evidence highlights the multisystemic nature of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Hepatic dysfunction is the primary extrapulmonary manifestation. In addition to the direct cytopathic effect of the virus, iatrogenic causes and genetic susceptibility are also postulated in the pathogenesis of hepatic damage in SARS-CoV-2 infection. Degree of liver toxicity in terms of altered biochemical indices were consistent with severity of coronavirus disease 2019 (COVID-19) illness and hospital stay. Hence, serial monitoring of hepatic indices in COVID-19 hospitalized patients may provide useful prognostic value to make timely corrective actions to avoid clinical deterioration.

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INTRODUCTION

Since the index case of coronavirus disease 2019 (COVID-19) infection was confirmed in the month of December 2019 in China[1], the upsurge of COVID-19 has led to devastating effects on global health[2]. Considering the incessant evolution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its impact on public health, SARS-CoV-2 variants were labelled as "variants of concern" (*e.g.,* Alpha, Beta, Gamma, Delta and Omicron) and "variants of interest" (*e.g.,* Eta, Iota, Kappa and Lambda) based on their attributes[3,4].

Each of the variants penetrates human cells by binding the cell surface receptor angiotensin converting enzyme (ACE) 2 *via* the spike protein subunit 1, while spike protein subunit 2 permits entry of the virus by enabling fusion of the virus envelope with the host cell membrane. This virus-cell fusion is facilitated by S protein priming by host cell proteases *viz* transmembrane protease serine 2 at a cleavage site (spike protein subunit 1/spike protein subunit 2), which is a polybasic furin cleft[3,4]. Fusion of the viral and cell membrane is followed by the entry of the virus inside the host cell to release the genetic material, *i.e.,* positive sense RNA. This RNA genome is the template for synthesis of new negative sense RNA with the help of RNA-dependent RNA polymerase. Newly synthesized RNA in turn facilitate the synthesis of positive sense RNA, which is responsible for the production of new cytoplasmic proteins, namely nucleocapsid protein and membrane protein. Nucleocapsid protein binds to freshly synthesized positive sense RNA, and membrane protein facilitates its assimilation into the endoplasmic reticulum (ER) to form nucleocapsids. These nucleocapsids are finally transferred to the cell membrane *via* the ER lumen and Golgi vesicle to the extracellular space *via* exocytosis[5,6].

These newly released virions infect the neighboring healthy cells and manifest COVID-19 with a diverse spectrum of symptoms, ranging from asymptomatic disease to severe symptoms, primarily associated with the respiratory system. However, emerging scientific evidence highlights the multisystemic nature of the disease, *i.e.,* involving extrapulmonary clinical manifestations such as myocardial infarction, neurological, ocular, dermatologic, gastrointestinal, kidney failure and liver dysfunction, owing to the tropism of the virus for ACE2 expressed in different human cells[7]. In fact, liver injury is the primary extrapulmonary manifestation, and the most common pattern is mild to moderate hepatocellular injury, observed in 14%-53% of the hospitalized patients with COVID-19. Furthermore, epidemiological studies have revealed that over one-half of infected patients with SARS-CoV-2 had deranged liver function tests characterized by abnormal levels of hepatic specific aminotransferases and other hepatic specific biochemical indices[8,9], while a small subset of patients was found with acute liver damage and fulminant hepatic failure[10,11]. Altered biochemical indices were more frequent in severely ill COVID-19 patients in contrast to patients presenting with mild to moderate illness[12,13].

In addition, certain therapeutic compounds can cause drug-induced liver injury (DILI)[14]. This was substantiated by the findings that toxicity was relieved after the cessation of these agents in *in vitro* and *in vivo* experiments[15,16]. These compounds include: (1) Antibiotics (azithromycin and ceftriaxone); (2) Antivirals [remdesivir (RDV), lopinavir (LPV)/ritonavir (RTV), favipiravir, umifenovir and triazavirin]; (3) Antimalarial [hydroxychloroquine (HCQ)]; (4) Adjuncts; (5) Steroids (dexamethasone); and (6) Immunomodulators [tocilizumab (TCZ)]. Overall, available data suggest that the spectrum of hepatic damage in SARS-CoV-2 infection may be accredited to the direct cytopathic effect of the virus through the ACE2 receptor, indirect involvement by systemic immune-mediated inflammation and by iatrogenic causes, *i.e.*, drug-induced[17,18].

Moreover, underlying genetic factors could also contribute to COVID-19-related liver abnormalities due to the occurrence in a subset of the patient population. In accordance with this, a substantial number of genetic-based and or association studies have addressed the genetic makeup of the host in regards to the predisposition to the development and progression of COVID-19-related liver injury to recognize the patient cohort for high clinical priority in terms of early or novel therapeutic interventions albeit with equivocal results.

Since there are limited data on the individual genetic susceptibility to SARS-CoV-2 infection-related liver abnormalities, a detailed understanding of the influence of specific genotypes will be crucial for clinical outcomes. In addition, substantial evidence from the scientific literature indicate that the degree of liver toxicity is due to a certain therapeutic regime employed in the treatment of SARS-CoV-2. Only a handful of researchers methodically and comprehensively explored the complete array of DILI in COVID-19 patients. Hence, it is worth reviewing the genetic and drug-induced perspectives on COVID-19-related liver injury. This review emphasized DILI in COVID-19 patients along with genetic insight into the development of SARS-CoV-2 infection-related liver injury by providing evidence from the most current pertinent publications using relevant keywords from online databases.

MATERIALS AND METHODS

Procedure adopted for relevant literature search

Using various electronic databases, namely Reference Citation Analysis, PubMed, China National Knowledge Infrastructure and Web of Science, our team carried out the relevant literature search using the following MeSH keywords: DRUG INDUCED LIVER INJURY AND COVID-19 OR DRUG INDUCED LIVER INJURY AND SARS-COV-2 OR DRUG INDUCED LIVER INJURY AND 2019 nCOV OR DRUG INDUCED LIVER INJURY AND CORONAVIRUS DISEASE with regards to drug-induced perspectives and COVID-19 AND LIVER INJURY AND POLYMORPHISM OR SARS-COV-2 AND LIVER INJURY AND POLYMORPHISM OR COVID-19 AND LIVER INJURY AND GENETIC INSIGHT OR SARS-COV-2 AND LIVER INJURY AND GENETIC INSIGHT for genetic insight of hepatic damage.

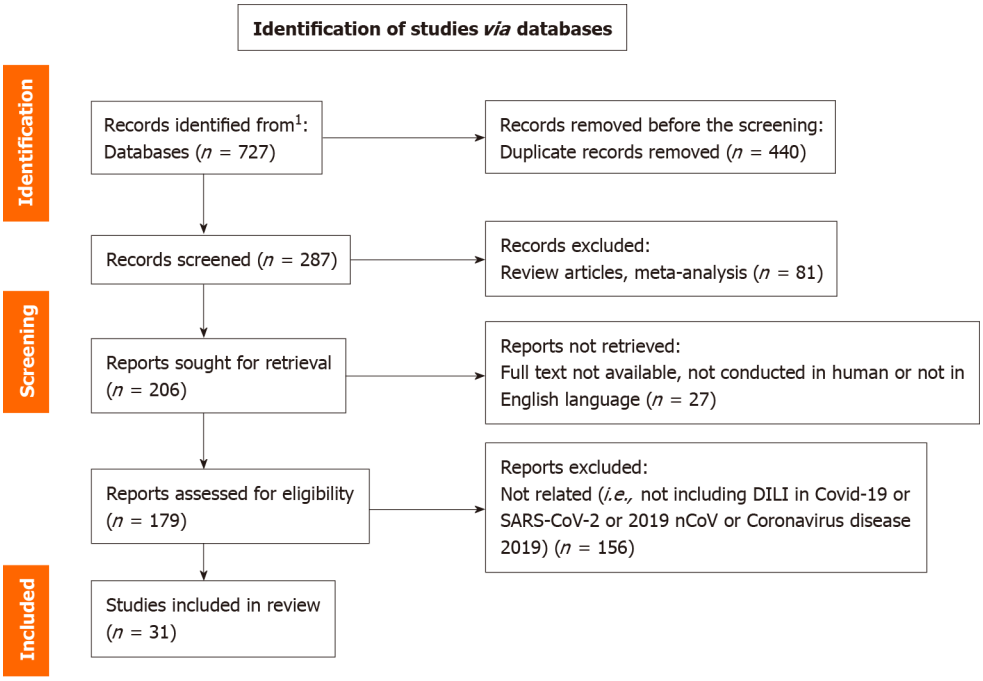
The criteria for inclusion were: Original articles; case series or reports; brief communication; or letters to the editor. However, articles were in English and published during between December 1, 2020 and April 30, 2022. The references of the articles from the initial search were screened to add any plausible relevant literature. However, studies with animal or cellular models were not included. Other criteria for exclusion were: Injury due to SARS-CoV-2 infection itself; and hepatic injury from herbal or dietary supplements. Finally, after eliminating duplicate articles, 31 (2 and 29 relating to genetic insight and DILI, respectively) out of 727 (14 and 713 articles, respectively, for genetic insight and DILI) articles were selected for review.

Document retrieval

By means of the aforementioned key words and in line with inclusion and exclusion criteria of the study, Sonagra AD, Dholariya S and Motiani A reviewed articles to ensure the fulfilment of inclusion criteria. Thereafter, Parchwani D and Singh R chose the articles to be finally included in the study. The authors then extracted the data: Author, site and sample size of study, stages of COVID-19, severity of disease, medication, outcome and/or DILI. All the mentioned information were extracted by standardized data extraction tables in duplicate.

RESULTS

A total of 8 studies on genetic insight and 279 studies concerning DILI were screened after removing the duplicate publications. Among the included studies, a total of 31 studies were considered suitable for the qualitative synthesis comprising 2 studies regarding genetic insight and 29 studies regarding DILI. Extraction of research articles (Figure 1) were performed as per the guideline prescribed in PRISMA statement 2020 and was done according to the published protocol (PROSPERO ID: CRD42022311838).



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Figure 1 Selection process of research studies from various databases according to PRISMA 2020. [1]Reference Citation Analysis, PubMed, China National Knowledge Infrastructure and Web of Science. SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; DILI: Drug-induced liver injury; COVID-19: Coronavirus disease 2019.

Hepatoxicity of commonly used drugs used in the treatment of SARS-CoV-2

Medications and or therapies employed in COVID-19 management, such as RDV, LPV/RTV, ribavirin, TCZ, hydroxyl chloroquine sulfate, *etc* are potentially hepatotoxic, specifically in high doses[19], and their administration in the form of polypharmacy exponentially increases the risk of DILI. The hepatotoxicity information as described across the included studies are compiled herewith for most frequently used drugs in the therapeutic regime of COVID-19. Table 1 depicts the relevant information per study. The most frequently associated drugs with DILI were RDV, LPV/RTV, TCZ, HCQ (+/-) azithromycin, ceftriaxone, paracetamol and enoxaparin.

RDV: A broad-spectrum nucleotide analogue prodrug, primarily used for hospitalized patients with COVID-19 is known to inhibit viral RNA polymerases. RDV had the maximum DILI rate/administration. Screening of the World Health Organization safety reports database revealed a total of 387 adverse drug reactions (ADRs) reports of RDV by late 2021. Out of which the majority were hepatobiliary (61%) followed by 34% hepatic. The most common documented adverse liver outcome in different studies were elevated hepatic specific aminotransferase in the range of 15%-50%, hypoalbuminemia and hyperbilirubinemia[20]. Levels of aminotransferase elevation were more prominent in seriously ill patients, suggesting a possibility of occurrence of various adverse events due to severity and gravity of SARS-CoV-2 infection. Furthermore, studies also stated that in a subset of patients RDV treatment was discontinued on account of the abnormally high liver aminotransferase levels[20]. Chew *et al*[21] conducted a study in a sample of 834 COVID-19 hospitalized patients and reported that 12.6% (*n* = 105) of patients showed a > 5 upper limit of normal (ULN) of serum aspartate aminotransferase. Among the adverse lung events, TCZ and RDV were significantly associated with DILI on univariate analysis. Further, Delgado *et al*[22] conducted a retrospective observational study for the assessment of DILI by a pharmacovigilance program using laboratory signals. Out of 8719 patients admitted for COVID-19, 4.9% of patients developed DILI. The drugs commonly associated with DILI were HCQ, azithromycin, TCZ and ceftriaxone. Out of these, RDV had the highest incidence rate of 992.7 DILI per 10000 defined daily doses.

These adverse events were further corroborated by individual case reports/series. In one such report [23], after 2-d administration of RDV, a sharp elevation in the level of alanine aminotransferase (ALT) was observed, which was instantly corrected after discontinuing RDV. Correspondingly, in other reported cases[24,25], increased levels of liver enzymes were found in patients on RDV +/- HCQ, who were initially treated with LPV/RTV. In a case series reported in the United States, Carothers *et al*[26] suggested that administering acetyl cysteine had a positive impact on the overall health of the patient and reversed acute liver failure due to RDV with hepatic specific transaminase (ALT and aspartate aminotransferase) levels > 5000 IU/L and increased total bilirubin levels (3.1 mg/dL), serum ammonia (161 μmol/L) and international normalized ratio of 2.3.

Table 1 Drug induced hepatoxicity outcome and other relevant information in the included studies

Sr. No.	Ref.	Type of study	Location	Number of study participants	Medication	Outcome
1	Grein *et al* [20], 2020	Cross sectional/follow-up study	United States, Canada, Europe, Japan	53	Remdesivir	Elevation of liver enzymes in 12/53 patients
2	Goldman *et al* [76], 2020	Randomized, open-label, phase 3 trial	United States, Italy, Spain, Germany, Hong Kong, Singapore, South Korea, Taiwan	397	Remdesivir	Elevated ALT in 26; elevated AST in 23; elevated bilirubin in 5
3	Wang *et al*[1], 2020	Randomized, double-blind, placebo-controlled, multicenter trial	China	158	Remdesivir	Elevated ALT in 2; elevated AST in 7, elevated bilirubin in 16, decreased albumin in 20
4	Antinori *et al* [77], 2020	Prospective (compassionate), open-label study	Italy	35	Remdesivir	Elevated transaminase in 15; elevated bilirubin in 7
5	Leegwater *et al*[23], 2021	Case study	Netherlands	1	Remdesivir	Elevated ALT, AST, ALP and GGT
6	Lee *et al*[24], 2020	Case series	South Korea	10	Remdesivir	Elevated ALT in 5; elevated AST in 5
7	Zampino *et al* [25], 2020	Case series	Italy	5	Remdesivir	Elevated ALT in 4; elevated AST in 4
8	Carothers *et al*[26], 2020	Case series	United States	2	Remdesivir	Elevated ALT, AST, bilirubin, INR, ammonia in both patients; elevated ALP in 1
9	Sun *et al*[27], 2020	Active monitoring study by hospital pharmacovigilance system	China	217	LPV/ritonavir, umifenovir	Elevated ALT in 30
10	Fan *et al*[28], 2020	Retrospective, single-center study	China	148	LPV/ritonavir	Elevated ALT in 27; elevated AST in 32; elevated GGT in 26; elevated ALP in 6; elevated bilirubin in 18
11	Cai *et al*[9], 2020	Cross-sectional study	China	417	LPV/ ritonavir, oseltamivir, interferon, NSAIDs, ribavirin	Elevated ALT in 167; elevated AST in 137; elevated GGT in 143; elevated ALP in 71; elevated bilirubin in 196
12	Jiang *et al* [29], 2020	Multicenter, retrospective, observational study	China	131	LPV/ritonavir	Elevated ALT in 45; elevated AST in 4; elevated bilirubin in 43
13	Serviddio *et al* [30], 2020	Case series	Italy	7	LPV/ritonavir, HCQ, azithromycin	Elevated ALT, AST, GGT in all patients
14	Guaraldi *et al* [32], 2020	Retrospective cohort study	Italy	179	Tocilizumab	No elevation of ALT or bilirubin was noted
15	Muhović *et al* [33], 2020	Case report	Montenegro	1	Tocilizumab	Elevated ALT and AST
16	Hundt *et al* [34], 2020	Retrospective observational cohort study	United States	1827	LPV/ritonavir, HCQ, remdesivir, tocilizumab	Elevated ALT in 1080 out of 1753; elevated AST in 1465 out of 1756; elevated ALP in 399 out of 1754; elevated total bilirubin in 284 out of 1747
17	Kelly *et al* [35], 2021	Retrospective analysis of hospitalized patients	Ireland	82	HCQ, azithromycin	Elevation of LFTs of more than ULN after 5 d therapy in 51/85 patients
18	Falcão *et al* [36], 2020	Case report	Brazil	1	HCQ	Elevated ALT and AST with normal bilirubin and GGT
19	Yamazaki *et al*[38], 2021	Case report	Japan	1	Favipiravir	Elevated ALT, AST, ALP, total bilirubin and LDH
20	Aiswarya *et al* [49], 2021	Observational prospective study	India	48	Remdesivir	No significant change in serum transaminases and LDH

22	Kaur *et al* [78], 2022	Case study	India	1	Remdesivir	Elevated ALT, AST and total bilirubin
23	Gao *et al*[79], 2022	Retrospective study	China	4010	Oseltamivir, arbidol, interferon, ribavirin, LPV/ritonavir, HCQ/CQ, antibiotics, antifungals, corticosteroids	395 out of 4010 developed DILI. 293 out of 395 received antibiotics, 25 out of 395 received antifungal, 42 out of 395 received oseltamivir, 52 out of 395 received ribavirin, 51 out of 395 received LPV/ritonavir, 47 out of 395 received interferon, 200 out of 395 received corticosteroid, 226 out of 395 received arbidol, 18 out of 395 received HCQ/CQ
24	Naseralallah *et al*[80], 2022	Retrospective study	Qatar	72	Azithromycin, HCQ, LPV	Elevated ALT and AST was implicated in 24 patients due to azithromycin, in 11 patients due to HCQ and in 11 patients due to LPV
25	Chew *et al* [21], 2021	Retrospective study	United States	834	Tocilizumab, remdesivir	105 out of 834 (12.6%) had elevated AST
26	Delgado *et al* [22], 2021	Retrospective observational study	Spain	8719	Remdesivir, hydroxy-chloroquine, azithromycin, tocilizumab and ceftriaxone	4.9% of 8719 patients developed DILI. Out of which remdesivir had the highest incidence of DILI per 10000 defined daily doses
27	Durante-Mangoni *et al* [81], 2020	Case report	Italy	4	Remdesivir	3 out of 4 patients had elevated AST and ALT
28	Wong *et al* [82], 2022	Self-controlled case series study	China	860	Remdesivir	334 (38.8%) out of 860 had acute liver injury
29	Montastruc *et al*[83], 2020	Multicenter study	France	387	Remdesivir	130 (34%) out of 387 developed liver injury

ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; DILI: Drug induced liver injury; HCQ: Hydroxychloroquine; LPV: Lopinavir; LDH: Lactate dehydrogenase; GGT: Gamma-glutamyl transferase; ULN: Upper limit of normal; INR: International normalized ratio; LFT: Liver function test; NSAID: Nonsteroidal anti-inflammatory drug.

LPV/RTV: Among the therapeutic regimes of COVID-19, LPV/RTV is one of the most common contributors of the liver ADRs. A study of 217 patients reported that LPV/RTV was found to be associated with 63% of total ADRs, while other drugs (umifenovir, chloroquine and antibacterial drugs) contributed to 47% of ADRs[27]. Correspondingly, a study of 148 patients reported that 48% developed hepatic function abnormality after admission to the hospital[28]. They emphasized that among such patients, 57.8% of the patients were treated with LPV/RTV. In another study, the authors reported an abnormal liver function in 12.1% of patients with the addition of each collateral medication[29]. Moreover, combined use of LPV/RTV with arbidol (umifenovir) in patients who were not terminally ill had an elevated risk of liver injury up to 3.58 times in comparison to those patient cohorts whose treatment regimen did not include LPV/RTV. It was postulated that RTV, being an inhibitor of chromosome 3 gene cluster A4, could promote hepatic toxicity from azithromycin *via* drug-drug interactions (polypharmacy)[26]. Similarly, metabolic interactions between the two medications [LPV/RTV and arbidol (umifenovir)] were studied *in vitro* using human liver microsomes by Serviddio *et al*[30] and concluded that LPV/RTV significantly impedes arbidol metabolism ($P < 0.005$), which may be the cause of DILI.

In a study with 163 mild and 29 severe patients with COVID-19, the multivariate analysis suggested RTV as one of the independent risk factors (odds ratio = 4.75, 95% confidence interval: 1.89-16.55, $P < 0.001$) in COVID-19 patients with liver injury[31]. In contrast to most of the studies that reported moderate-to-severe elevations in serum aminotransferase levels in patients under LPV/RTV treatment, Cai *et al*[9] reported increased odds of liver injury by four-fold in the LPV/RTV treated group among the enrolled 417 COVID-19 patients in China. The most significant increases were gamma-glutamyl transferase activity and total bilirubin. Because the drug was not efficacious, it was discontinued from the COVID-19 treatment regimen.

TCZ: TCZ, an interleukin-6 receptor antagonist monoclonal antibody, is primarily used for severely ill COVID-19 patients to arrest the cytokine storm. Guaraldi *et al*[32] did not find any adverse effects on the liver function test in a retrospective study involving 1351 COVID-19 patients treated with TCZ. In the following line of evidence, Serviddio *et al*[30] published a case series from Italy and displayed substantially altered hepatic and lung function tests after administration of LPV/RTV, HCQ and azithromycin for 5-7 d. These patients showed an improvement in both liver and lung function after the use of TCZ within 3 wk. However, the first case reported of DILI due to the use of TCZ did not deny the possibility of serious hepatotoxicity when used with other hepatotoxic drugs. In this case, 1 d after TCZ adminis-

tration, the levels of serum transaminase increased up to 40-fold (aspartate aminotransferase of 1076 IU/L and ALT of 1541 IU/L)[33].

Another retrospective cohort study with 1827 patients conveyed that a positive correlation exists with usage of RDV, LPV/RTV, HCQ and TCZ and hepatotoxicity[34]. They observed peak hospitalization liver transaminase elevations more than 5 times the ULN.

HCQ with or without azithromycin: Most cases of DILI were reported for HCQ after its emergency use authorization for COVID-19 infection. Kelly *et al*[35] conducted an analysis in two groups of 134 patients. One group was treated with HCQ/azithromycin, while the other group was devoid of this targeted therapy. They reported no significant difference in the liver function tests between the two groups. On the contrary, a 10-fold elevation in levels of liver transaminases in the serum after HCQ administration was reported by Falcão *et al*[36]. They revealed that serum levels of hepatic enzymes rapidly declined after the withdrawal of HCQ from the treatment regimen.

Corticosteroids: Systemic corticosteroids, mainly dexamethasone, are widely used in patients with SARS-CoV-2 infections. However, an independent basis of hepatoxicity is uncommon. They are associated with minor, self-limiting elevations in serum aminotransferase. A study (n = 1040 COVID-19 patients) reported that the administration of corticosteroids was found to be correlated (adjusted odds ratio = 3.9) with development of acute liver injury on an independent basis (95% confidence interval: 2.1-7.2)[16].

Enoxaparin: Enoxaparin is associated with minor, self-limiting elevations in serum aminotransferase, but values > 5 ULN are uncommon. Sporadic cases of mild increases in serum bilirubin and alkaline phosphatase have been reported[37].

Favipiravir: A single study observed adverse liver events after favipiravir administration[38]. Authors of the study reported a COVID-19 patient who manifested bacterial pneumonia as a complication of COVID-19 during his hospital stay. The therapeutic regime of the patient was LPV/RTV combined with interferon β-1b. Following administration of favipiravir, liver transaminases and total bilirubin increased suggesting a cholestatic liver injury. The liver injury, in this case, may have been triggered by antibacterial treatment, which may have further deteriorated by treatment with a high dose of favipiravir.

Tang *et al*[39] found 17.31% (n = 3425) of patients exhibited DILI in a cohort of 19782 COVID-19 patients. The odds ratio for DILI was 2.99 (2.59-3.46), 5.39 (4.63-6.26) and 3.16 (2.68-3.73) when comparing LPV-RTV with all other drugs, RDV and HCQ/chloroquine, respectively. A single-center, open-label, parallel-arm, stratified randomized controlled trial completed by Panda *et al*[40] observed DILI in the form of elevated liver enzymes in 2 out of 67 participants who received a high dose of ribavirin.

Genetic insight towards SARS-CoV-2-induced liver injury

In the matter pertaining to a genetic propensity towards SARS-CoV-2 induced liver injury, in the United Kingdom Biobank cohort[41] an elevated risk score of genetic fatty liver disease (FLD) based on glucokinase regulator, membrane bound O-acyltransferase domain containing 7, patatin like phospholipase domain containing 3 (*PNPLA3*) and transmembrane 6 superfamily 2 human gene genetic variants was not found to be associated with a higher probability of developing severe SARS-CoV-2. Hence, this finding challenges the causal role for metabolic-associated FLD in COVID-19 and implies that genetic susceptibility to hepatic fat deposition does not, in and of itself, increase the risk of developing a severe form of the disease[41]. However, contrary to this, membrane bound O-acyltransferase domain containing 7 rs641738[42], rs11385942 G>GA at chromosome 3 gene cluster and rs657152 C>A at the ABO blood locus were significantly associated with the severity of livery injury in admitted SARS-CoV-2 patients[43,44]. Thus, the genetic basis of SARS-CoV-2-induced liver injury is not yet fully understood, and additional research is required to validate the involvement of any specific variant form. Table 2 depicts the commonly employed therapeutic drugs for COVID-19, with its hepatic side effects and 'Likelihood Score' by the LiverTox database[45].

DISCUSSION

The primary analysis of this review revealed that DILI is due to the large-scale use of drugs/off-label drugs in the prophylactic and therapeutic regimen of COVID-19, and the causal relationship of genetic susceptibility with hepatic damage in SARS-CoV-2 infected patients is incomprehensible. Hepatic damage may arise either through intrinsic or idiosyncratic mechanisms. The intrinsic pathway is predictable and has a short latency period. However, COVID-19-related liver injury (drug-induced and/or genetic-based) predominantly follows the idiosyncratic mechanism, *i.e.*, it is unpredictable with a variable latency period[19].

Table 2 Hepatoxicity and likelihood score of therapeutic agents of coronavirus disease 2019

Drug	Hepatotoxicity	Likelihood score
Remdesivir	A duration of 7-14 d of administration caused elevation of serum aminotransferases up to > 5 times of ULN. Elevation of > 5 times ULN were reported in 9% of patients but returned to normal after discontinuation. Prolonged and more severe effects were seen in critically ill patients with multiorgan involvement, pre-existing comorbidities and who had received combination therapy with other hepatotoxic agents like amiodarone	D
Lopinavir/ritonavir	A greater degree of rise in serum aminotransferase levels (> 5 times ULN) is mostly seen in association with immunodeficiency states. The pattern varies from hepatocellular to cholestatic or mixed type. Discontinuation leads to the normalization of enzyme levels. However, severe cases of acute liver failure or end stage liver disease are also reported with re-exposure of the drug	D
Tocilizumab	Reported to cause mild elevation of aminotransferases commonly, that is usually transient and asymptomatic, but rare instances of liver injury manifesting as jaundice and reactivation of hepatitis B are seen. ALT elevation (1-3 times ULN) was seen in 10%-50% of patients, which returned to baseline within 8 wk after stopping treatment. No effect on bilirubin or ALP levels were seen	C
Hydroxychloroquine	Clinically apparent liver injury is rare. In clinical trials for COVID-19 prevention and treatment, there were no reports of hepatotoxicity, and serum enzyme elevation was also low	C
Corticosteroids	Long-term use and high doses can result in hepatomegaly and steatosis. Can also trigger or exacerbate pre-existing or co-existing conditions like NASH, viral hepatitis or autoimmune hepatitis. Serum aminotransferase levels can rise up to 10-40 times ULN	A
Enoxaparin	4%-13% of patients showed mild elevation in serum aminotransferase levels. Rapid onset of liver injury symptoms after starting the drug (within 3-5 d) but rapid recovery (1-4 wk) after discontinuation of therapy is seen	E
Favipiravir	Pretreatment with other hepatotoxic drugs like lopinavir/ritonavir and IF-β 1B lead to an increase in liver transaminase and bilirubin levels by manifold suggesting cholestatic injury. Isolated use is not known to cause any severe liver injury	D

A: Well known hepatotoxicity; B: Highly likely hepatotoxicity; C: Probable hepatotoxicity; D: Possible hepatotoxicity; E: Unlikely hepatotoxicity. COVID-19: Coronavirus disease 2019; ULN: Upper limit of normal; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; NASH: Nonalcoholic steatohepatitis.

Drugs like LPV/RTV were associated with moderate to severe elevation (> 5 ULN) of hepatic specific aminotransferases in serum and exhibited a significantly (4 times) higher chances of liver injury[9]. The degree of hepatic damage varies widely, *i.e.*, from injury of hepatocytes to complete stagnation of bile acid secretion (cholestatic injury) or may be both in certain cases[45]. Correspondingly, medication of COVID-19 patients with LPV/RTV might exaggerate dysfunction of hepatic cells in particular to hepatitis B virus and hepatitis C virus infection cases[46]. However, the efficacy of LPV/RTV in SARS-CoV-2 patients is not fully understood and requires further evaluation[47,48]. In contrast, a study in China suggested out that administration of antibiotics, ribavirin and nonsteroidal anti-inflammatory drugs is not associated with a statistically significant risk of hepatic damage[9].

Likewise, studies evaluating the ADRs of RDV reported that it could lead to liver injury, barring one [49]. Liver injury caused by RDV manifested only after the 3rd d of its administration as elevated hepatic specific transaminases, coagulopathy and hepatic encephalopathy. N-acetyl cysteine is recommended for the management of acute liver failure induced by RDV and discontinuation of drug for progression to acute liver failure[26]. It was suggested that the following criteria should be considered for an immediate cessation of RDV treatment: Elevation of ALT > 5 times ULN or elevation of alkaline phosphatase > 2 times ULN; increased level of total bilirubin more than > 2 times ULN; immediate incidence of coagulopathy; or in cases where the patient's condition is deteriorating[50]. Thus, to diminish RDV-induced toxic effects, assessment of liver status must be completed before drug initiation, and continuous monitoring of the liver function test should be performed during the course of treatment.

However, the most contentious reports were of TCZ. A retrospective cohort study[32] and meta-analysis[47,48] reported that TCZ by itself is not associated with liver injury in COVID-19 patients. One study reported that TCZ had a positive effect on clinical and laboratory parameters caused by the use of LPV/RTV[30]. ALT levels fall within the normal range from > 5 times ULN after administration of TCZ. On the other hand, a study conducted by Muhović *et al*[33] reported that the hepatotoxic effects of TCZ were increased in cases of prior administration of antiviral drugs (LPV/RTV).

DILI in COVID-19 patients is often dependent on numerous factors. For instance, co-existing medical conditions (porphyria cutanea tarda, viral hepatitis and rheumatologic diseases) could increase the risk of developing toxicity due to recommended drugs[36]. Further, drug-drug interaction (*e.g.*, chloroquine and its derivatives combined with anti-rejection immunosuppressants[51]) can lead to detrimental effects. For example, the prevalence of liver damage was 15.2% in a sample size of 208 COVID-19 patients on RDV only, whereas it was 37.2% among 775 patients treated with RDV and LPV/RTV[52], substantiating the concept of polypharmacy.

Among included studies, one study reported that the grade of hepatotoxicity was not statistically different between the controls and cases, who were treated with HCQ and azithromycin[35]. Nevertheless, divergent findings are also frequently reported for HCQ and has been hypothesized that the presence of pre-existing inflammation (mild to moderate) might increase the risk of liver damage by HCQ (+/- azithromycin) in the doses that are not hepatoxic due to the production of cytotoxic metabolites from drug metabolism by inflammatory cells with the help of myeloperoxidase enzyme. Ivermectin (anti-parasite medication) and colchicine (anti-inflammatory agent) are well-tolerated and have been reported to reduce the severity, length of hospital stay and prevention of a cytokine storm[47,53,54], but efficacy of these drugs in the management of SARS-CoV-2 infected patients is still not fully understood[55,56]. Hepatotoxic effects are not well documented.

Mechanisms of DILI

Drugs employed in the management of SARS-CoV-2 infection (RDV, LPV/RTV, ribavirin, TCZ, HCQ or any other drugs) are metabolized by the hepatic cells. Liver damage with an associated increase of hepatic specific indices is predictable and has been corroborated and cited in the scientific literature[57-61] (Table 3).

Critical biochemical properties of anti-COVID-19 drugs that might lead to hepatotoxicity in susceptible hosts are lipophilicity, mitochondrial liability, generation of cytotoxic metabolites, their metabolic pathway in the liver and the ability to inhibit hepatic transporters[62]. Patients who died with severe COVID-19 had moderate microvesicular steatosis, a condition characterized by a variant form of hepatic fat accumulation and modest lobular and portal activity in their liver biopsies, suggesting that the liver injury may have been due to either viral- or drug-induced mechanisms. Steatosis in lieu of drugs occurs due to interference with β-oxidation of fatty acids, oxidative phosphorylation or both by certain drugs[63], resulting in the accumulation of free fatty acids, which are converted to triglycerides [64].

Clinical and murine studies have provided evidence that pre-existing medical conditions, *e.g.*, inflammatory diseases, increased blood pressure and diabetes mellitus, augment SARS-CoV-2 hepatic injury, possibly because of ACE inhibitors or angiotensin receptor blockers, which results in ACE2 upregulation[65,66]. The presence of pre-existing nonalcoholic FLD sensitizes hepatocytes to antipyretic agents containing acetaminophen[67].

Reduced and/or suppressed activity of the CYP family or cytochrome P450 (enzyme responsible for metabolism of xenobiotics) is also a plausible mechanism to alter the activity of liver cells[68]. CYPs are downregulated due to repressive effects exerted by interleukins and cytokines, which are upregulated during COVID-19 infection, leading to toxicity of several COVID-19 drugs[68]. Drug-drug interactions also play an important role in the development and progression of DILI, as exemplified by the clearance of umifenovir, which is compromised by concomitant use of LPV/RTV due to its inhibitory effect on cytochrome P3A[29].

A precedent of hepatic transporter inhibition by COVID-19 drugs to manifest the liver injury is reported by many studies with regard to LPV, a prominent blocker of multidrug resistance-associated protein-2. A study performed on rats reported the accumulation of taurocholic acid inside the liver cells following the 10-min exposure of rat liver cells to protease inhibitor (PIs) drugs, LPV and RTV, indicating that the Pis inhibit the efflux of bile salts from liver cells[69]. Experimental studies showed the inhibitory effect of Pis on multidrug resistance-associated protein-2[70,71]. Holmstock *et al*[70] also reached a similar conclusion using 5(6)-carboxy-2′,7′-dichlorofluorescein through confocal imaging. Another recent study by Khalatbari *et al*[72] focused on oxidative stress damage leading to hepatotoxicity and FLD due to Pis, LPV and RTV. They reported the interference of these drugs by ER-Golgi trafficking through inhibition of Ras converting CAAX endopeptidase-1 and any of its substrate, which in turn leads to development of fatty liver and cellular stress.

Additionally, exhaustion of P450 activity to metabolize large and multiple amounts of COVID-19 drugs as a treatment regimen can also be the cause of hepatoxicity. Simultaneously, studies reported that administration of certain drugs (LPV/RTV) assists in the reactivation of hepatitis B and C viruses and results in hepatoxicity[73]; administration of HCQ in patients with porphyria cutanea tarda leads to significant liver damage[73] due to the interaction of reactive metabolites of HCQ and the inflammatory response due to SARS-CoV-2 infection[74].

However, there is a deficit of uniformity and standardization of DILI due to a lack of reliable and exclusive evidence pointing towards the drugs used in the treatment of COVID-19. Moreover, there is considerable overlap and commonality in the presenting symptoms of hepatic damage due to COVID-19 infection per se and due to drugs given for its treatment. Increased vigilance on the part of the clinicians is warranted so that cases of severe liver damage suspected to be caused by the drugs can be reported and entered into the National/International database. The *R* value can be considered as a diagnostic approach for the pattern of liver injury (*i.e.*, $R > 5$ is considered hepatocellular DILI, $R < 2$ is considered cholestatic DILI, and $R = 2\text{-}5$ is considered mixed DILI; *R* value = ALT value/ULN divided by alkaline phosphatase value/ULN).

Table 3 Mechanism of hepatic damage in severe acute respiratory syndrome coronavirus 2 infection

Proposed main mechanism	Explanation
Direct cytotoxicity	Active SARS-CoV-2 replication in hepatic cells, which further binds to hepatic and biliary epithelial cells by angiotensin-converting enzyme 2 and damage them by direct infection[57]
Immunological damage	Severe inflammatory response generated by SARS-CoV-2 further damages hepatic cells by immune mediated pathogenesis[58]
Drug-induced	Antiviral drugs such as remdesivir, chloroquine and ritonavir are possibly hepatotoxic[59]
Reactivation of pre-existing liver illness	Increased risk to develop hepatotoxicity in the presence of pre-existing liver diseases. In addition, baricitinib also causes reactivation of hepatitis B virus infection[60]
Anoxia	Anoxia or hypoxia leads to respiratory failure in SARS-CoV-2, which further leads to hypoxic hepatitis[61]

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

Genetic aspect of hepatic consequences of SARS-CoV-2 infection

Irrespective of the aforementioned drugs in the treatment regime, Machill *et al*[42] reported that patients with carrier genotypes of membrane bound O-acyltransferase domain containing 7 rs641738 polymorphism had significantly elevated bilirubin, ALT and alkaline phosphatase levels and decreased serum albumin levels during hospitalization. This points towards genetic susceptibility.

Dongiovanni *et al*[75] explored the polygenic risk score of hepatic fat content and genetic markers of liver fibrosis (PNPLA3 I148M variant). Both polygenic risk score of hepatic fat content and PNPLA3 I148M were found to be inherited independently of dysmetabolism at conception to gain a better understanding into the relationship between FLD, liver damage and COVID-19[23]. They reported that rs11385942 G>A at chromosome 3 gene cluster and rs657152 C>A at the ABO blood locus were significantly associated with the severity of liver injury in admitted SARS-CoV-2 patients[75]. In fact, although a greater ALT was related to a genetic propensity to FLDs during SARS-CoV-2, this was accompanied by reduced systemic inflammation or C-reactive protein levels and maintenance of hepatic production or circulating serum albumin levels in carriers with the PNPLA3 I148M variant. The protective impact of the non-secretor ABO phenotype against SARS-CoV-2 infection has yet to be explained; it depends on whether or not differences in membrane glycan shedding underlie differential tissue susceptibility such as the liver and lungs. Finally, it was observed that the risk of severe COVID-19 in hospitalized patients was not elevated by the use of genetics-based assessment, which is a reliable unconfounded all-time proxy of a tendency to and progression of FLD. Therefore, genetic propensity to obtain liver fat, despite aiding in liver injury, may unexpectedly defend against inflammation throughout SARS-CoV-2, suggesting that FLD predilection does not automatically lead to increased inflammation[43].

To summarize, the available evidence outlines that the degree of lipophilicity of drugs, inflammatory response to the antivirals, metabolization by CYP3A4 in the liver, interference of drugs with various transporters in the liver and molecules/proteins accountable for protection against the xenobiotics (*e.g.*, organic anion transporting polypeptide 1B1, p-glycoprotein, multidrug resistance-associated protein-2, breast cancer resistance proteins and ER-Golgi trafficking primarily by inhibiting Ras converting CAAX endopeptidase-1) are the underlying factors responsible for drug hepatotoxicity in SARS-CoV-2 infection treatment. Concurrently, drugs have a detrimental effect on bile salt export pump activity (an outflow transporter system responsible for excretion of waste and foreign substances from the hepatic cells) and thus becomes a central factor in the cholestasis process. In addition, robust shreds of evidence are lacking regarding the genetic predisposition to hepatic dysfunction in SARS-CoV-2 infection. Larger prospective studies are warranted in this regard.

CONCLUSION

Hepatic dysfunction in SARS-CoV-2 infection could be the result of an individual drug or due to interactions among more than one drug and may include a subset of the patient population that has a genetic propensity. Thus, serial estimation of hepatic indices in SARS-CoV-2 hospitalized patients, especially patients on treatment with drugs like RDV, LPV/RTV, favipiravir, HCQ and TCZ should be performed to take corrective actions for iatrogenic causes to avoid clinical deterioration.

Limitations

Nonetheless, our findings described here are only an assortment of studies and do not imply causation. Other limitations include the sample sizes that were comparatively small. The methodology adopted in

the included studies had a wide variation (as few studies only raised the probability of DILI rather than confirming the role of drugs with certainty). The therapeutic regimen, duration difference in the samples collected after hospital admission, the failure to record or the variation of the onset of disease/degree of liver injury and discrepancy in correcting different clinic and biochemical indices (sex, co-existing morbidities and age) varied between studies. The discrepancy in the measurement of liver indices and sub-classification of the cases was another limitation. Finally, only articles published in English were considered for analysis, which may have a local literature bias. In spite of all the aforementioned confines, the present review detailed important systematic data on the genetic susceptibility of liver damage and DILI in SARS-CoV-2 infection.

ARTICLE HIGHLIGHTS

Research background

Available data advocate that the spectrum of hepatic damage in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection may be accredited to the direct cytopathic effect of the virus, indirect involvement by systemic immune-mediated inflammation and by iatrogenic causes, *i.e.*, drug induced. Empirical use of potentially hepatotoxic drugs in the management of SARS-CoV-2 infection is considered as one of the major etiopathogenetic factor for liver injury. Moreover, experimental and clinical evidence has shown that an underlying genetic factor may also be present. Hence, it is important to understand the genetics and iatrogenic-based mechanisms for liver dysfunction to make timely remedial measures.

Research motivation

To identify drug-induced liver injury in coronavirus disease 2019 (COVID-19) patients along with a genetic insight for the development of SARS-CoV-2 infection related liver injury to provide better care and timely management of critical patients.

Research objectives

To explore drug-induced and genetic perspectives in the development of SARS-CoV-2 infection related liver injury.

Research methods

A systematic literature search was carried out in multiple electronic databases: PubMed, Reference Citation Analysis, China National Knowledge Infrastructure and Goggle Scholar. The literature was screened using related MeSH keywords and relevant data. The inclusion criteria were English language articles published between December 1, 2020 and April 30, 2022. Reference lists from the articles in the initial search were screened to identify additional literature. There was no exclusion based on the study outcome and stage or severity of SARS-CoV-2 infection. However, studies with animal or cellular models were not included. Other criteria for exclusion were: injury due to SARS-CoV-2 infection itself; and hepatic injury from herbal or dietary supplements.

Research results

The primary analysis of this review revealed that DILI was due to the large-scale use of drugs/off-label drugs in the prophylactic and therapeutic regimen of COVID-19, and the genetic susceptibility underlying liver damage in COVID-19 patients is not yet fully understood. COVID-19-related liver injury (drug-induced and/or genetic-based) predominantly follows the idiosyncratic mechanism, *i.e.*, it is unpredictable with a variable latency period. In most commonly used drugs, the hepatic specific aminotransferases and other biochemical indices were elevated and were significantly associated with severity, hospital stay, number of COVID-19 treatment drugs and worse clinical outcomes.

Research conclusions

Hepatic dysfunction in SARS-CoV-2 infection could be the result of individual drugs or due to drug-drug interactions and may include a subset of the patient population with a genetic propensity. Thus, serial estimation of hepatic indices in SARS-CoV-2 infection hospitalized patients should be performed to make timely corrective actions for iatrogenic causes to avoid clinical deterioration.

Research perspectives

Additional prospective studies are warranted in this regard to justify drug-induced liver injury due to COVID-19 treatment along with the genetic predisposition, which should provide optimization of disease status.

FOOTNOTES

Author contributions: Sonagra AD, Dholariya S and Motiani A contributed to the search and examination of articles, analysis, manuscript writing and proof reading; Parchwani D designed the research study, examined articles and wrote the manuscript; Singh R contributed to literature search and analysis; and all authors have read and approved the final manuscript.

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REVIEW

Intestinal barrier dysfunction as a key driver of severe COVID-19

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Abstract

The intestinal lumen harbors a diverse consortium of microorganisms that participate in reciprocal crosstalk with intestinal immune cells and with epithelial and endothelial cells, forming a multi-layered barrier that enables the efficient absorption of nutrients without an excessive influx of pathogens. Despite being a lung-centered disease, severe coronavirus disease 2019 (COVID-19) affects multiple systems, including the gastrointestinal tract and the pertinent gut barrier function. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can inflict either direct cytopathic injury to intestinal epithelial and endothelial cells or indirect immune-mediated damage. Alternatively, SARS-CoV-2 undermines the structural integrity of the barrier by modifying the expression of tight junction proteins. In addition, SARS-CoV-2 induces profound alterations to the intestinal microflora at phylogenetic and metabolomic levels (dysbiosis) that are accompanied by disruption of local immune responses. The ensuing dysregulation of the gut-lung axis impairs the ability of the respiratory immune system to elicit robust and timely responses to restrict viral infection. The intestinal vasculature is vulnerable to SARS-CoV-2-induced endothelial injury, which simultaneously triggers the activation of the innate immune and coagulation systems, a condition referred to as "immunothrombosis" that drives severe thrombotic complications. Finally, increased intestinal permeability allows an aberrant dissemination of bacteria, fungi, and endotoxin into the systemic circulation and contributes, to a certain degree, to the over-exuberant immune responses and hyper-inflammation that dictate the severe form of COVID-19. In this review, we aim to elucidate SARS-CoV-2-mediated effects on gut barrier homeostasis and their implications on the progression of the disease.

Key Words: COVID-19; SARS-CoV-2; Intestinal barrier; Dysbiosis; Immunothrombosis; Gut-lung axis

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Core Tip: Severe coronavirus disease 2019 (COVID-19) is associated with a multi-layered disruption of gut barrier integrity. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) inflicts direct cytopathic or indirect immune-mediated injury to intestinal epithelial and endothelial cells and enhances paracellular permeability by downregulating tight junction proteins. SARS-CoV-2 induces profound gut microbiome alterations accompanied by dysregulation of mucosal immune responses. Gut dysbiosis attenuates, through the gut-lung axis, the ability of the respiratory immune system to elicit vigorous responses to contain SARS-CoV-2. Additionally, intestinal barrier dysfunction promotes endothelial activation and predisposes to detrimental COVID-19-related thrombotic complications. Finally, bacterial translocation and endotoxemia contribute to over-exuberant immune responses and hyper-inflammation in severe COVID-19.

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INTRODUCTION

The emergence of the novel, pathogenic, and highly transmissible severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19), triggered an unprecedented public health crisis with profound socioeconomic sequelae. In most cases, COVID-19 is associated with mild-to-moderate symptoms that mainly involve the respiratory tract. However, in a subset of patients, COVID-19 may progress into a more severe disease plagued with complications such as pneumonia, acute respiratory distress syndrome (ARDS), coagulopathy, myocarditis, hepatic injury, renal dysfunction, sepsis, multiple organ failure, or even death[1]. These detrimental effects are considered to be driven by aberrant activation of the host's immune system in response to viral invasion and proliferation into the pulmonary parenchyma[2]. In particular, the virus-laden pneumonocytes secrete excessive amounts of pro-inflammatory mediators and chemoattractant molecules, such as interleukins (IL-1β, IL-6, IL-7, IL-8, IL-12), tumor necrosis factor alpha (TNF-α), interferons (IFN-γ, IFN-λ), macrophage inflammatory protein-1 alpha (MIP-1α), monocyte chemoattractant protein-1 (MCP-1), and granulocyte colony stimulating factor. Subsequently, the recruitment and activation of innate and adaptive immune cells into the lungs further aggravate tissue injury and inflammation[2]. In parallel, the systemic dissemination of this "cytokine storm" precipitates overactivation of the immune system beyond the sites of infection and elicits hyperinflammatory responses that impair the function of several organs including the heart, kidneys, liver, nervous system, and gastrointestinal (GI) tract[3].

The GI system harbors an enormous interface that directly communicates with the external environment of the body and fulfills multifaceted functions. The GI mucosa serves as a semi-permeable membrane, allowing the efficient absorption of water, electrolytes, and nutrients while, in parallel, preventing the influx of xenobiotics, intraluminal microbiota, microbial components, or other inflammatory stimuli into the organism[4]. This subtle balance is maintained thanks to intestinal barrier function, which, apart from providing a physical barrier, regulates complex immune system responses and mediates the intricate crosstalk with the gut microbiome[5]. The integrity of the intestinal barrier can be compromised in many acute or chronic pathological conditions, leading to increased bacterial translocation and excessive penetration of pro-inflammatory signals. This dysfunction is associated with infectious complications and the establishment of a systemic pro-inflammatory status that can exacerbate or accelerate the pathophysiological processes of the underlying disease[6].

In this review, we summarize the deleterious effects of SARS-CoV-2 infection on gut barrier homeostasis. Subsequently, we discuss the mechanisms that explain how intestinal barrier dysfunction might drive severe COVID-19 or induce detrimental complications.

GI INVOLVEMENT AND THE MECHANISMS OF INTESTINAL INFECTION IN COVID-19

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus with a genome of approximately 30000 nucleotides that encodes 29 proteins, including 4 structural proteins: spike (S), nucleocapsid (N), membrane (M), and envelope (E) proteins. The S protein consists of the S1 subunit, which interacts with angiotensin-converting enzyme 2 (ACE2), and the S2 subunit which promotes membrane fusion[7,8]. The principal route of transmission of SARS-CoV-2 is *via* respiratory droplets or aerosols

from person-to-person, and its main target is type II alveolar epithelial cells (AEC2s). The entry of SARS-CoV-2 is primarily mediated by the attachment of the S glycoprotein with ACE2 on the cellular surface, a process facilitated by proteolytic cleavage at the S1/S2 boundary by host proteins. Transmembrane protease serine 2 (TMPRSS2) is a significant host protease that mediates the cleavage of S2 protein, leading to conformational changes that precipitate viral entry through membrane fusion[7, 8]. Intriguingly, ACE2 and TMPRSS2 are widely co-expressed on the membrane of intestinal epithelial cells (IECs) at a level comparable or even higher to that of the AEC2s, rendering the GI tract a potential target for SARS-CoV-2 infection[9].

Multiple studies have demonstrated that the development of GI-related symptoms is a common extrapulmonary manifestation, affecting up to one-fifth of patients with COVID-19[10-12]. According to a meta-analysis of 108 studies comprising 17776 COVID-19 patients, the pooled prevalence estimates of GI symptoms were: 21% for loss of appetite, 13% for diarrhea, 8% for nausea or vomiting, and 4% for abdominal pain, while derangement of liver function tests was observed in 24% of the participants[10]. Whether the occurrence of GI symptoms is associated with COVID-19 severity or outcomes has been a field of debate. Most meta-analyses support that GI-symptoms and predominantly abdominal pain tend to appear at higher rates among patients with severe disease[10,13-15]; however, these symptoms are not always predictive of mortality or intensive care unit (ICU) admission[16-18]. Importantly, viral RNA can be detected in the stool of about half of COVID-19 patients and, especially, in those with GI-related symptoms[19,20]. Viral rectal shedding appears to persist long after RNA clearance in respiratory samples (mean duration: 21.8 d *vs* 14.7 d)[20], while RNA concentration in the stool may be as high as 10^7 copies/g, exceeding even that in pharyngeal swabs in some cases[21]. The combination of this particularly high RNA load in the stool and the long-lasting viral presence in fecal samples strongly suggests that SARS-CoV-2 invades and proliferates in the intestinal tract. Indeed, COVID-19 elicits acute inflammatory responses in the gut, as documented by increased fecal calprotectin levels[22]. Accordingly, Livanos *et al*[18] provided direct evidence of direct infection of IECs by SARS-CoV-2 *in vivo*, using immunofluorescence staining and electron microscopy. Viral subgenomic mRNA (sgmRNA) is transcribed in infected cells, but is not encapsulated into virions, which means that sgmRNA is vulnerable to enzymatic degradation by intestinal ribonucleases. Therefore, the detection of sgmRNA in the stool of COVID-19 patients also indicates that the virus can actively and specifically replicate in the gut[21].

Although the data above corroborate the concept of SARS-CoV-2 tropism for the GI system, the routes and mechanisms of infection remain less clarified. At least theoretically, the feasibility of the fecal-oral route of transmission is supported by prolonged viral excretion in the feces. Nevertheless, although SARS-CoV-2 retains viability in stool for 1-2 d at room temperature, this is accompanied by a profound 5-log loss of its infectivity[23]. In addition, ingested SARS-CoV-2 needs to overcome the harsh gastric acidity, resist the detergent effects of bile acids in the duodenum, and avoid degradation by digestive enzymes to preserve its infectivity and spread into the intestinal epithelia[24]. The uninterrupted function of these host defense mechanisms hinders the fecal-oral transmission of enveloped viruses, such as SARS-CoV-2[25]. Besides, several lines of evidence support that intestinal infection by SARS-CoV-2 could occur *via* the bloodstream[19,26]. The virus replicating in pulmonary alveolar epithelial cells or in ciliary cells of the nasal cavity is capable of breaching the basement membrane and causing viremia[27]. The GI tract comprises an extended network of capillaries harboring vascular epithelial cells, which are potential targets of circulating SARS-CoV-2. Thereafter, the proliferating virus is released from the infected cells into the gut, where it can infect neighboring enterocytes or reenter the circulation to generate new cycles of infection[27,28].

THE INTESTINAL BARRIER FUNCTION IN HEALTH

The gut barrier function is equipped with three major lines of defense that conjointly serve its complex purpose[5]. First, the mechanical barrier consists of tightly linked columnal IECs, the overlying mucus layer, and the capillary endothelial cells of the submucosa[29]. Tight junctions (TJs) and adherens junctions (AJs) are transmembrane multiprotein complexes that confer structural stability to the mechanical barrier and engender the establishment of cell polarity. TJs connect the most apical of the lateral surfaces of the adjacent epithelial or endothelial cells (kissing points) and form channels that regulate the selective diffusion of ions and salutes through the paracellular space. AJs are more basal than TJs and contribute to the establishment and maintenance of cell-cell adhesion[30,31]. The mucus layer comprises a hydrated network of polymers, predominantly highly glycosylated mucin proteins, as well as several immune regulators, such as antimicrobial proteins (AMPs) and secretory immunoglobulin A molecules. The mucus lubricates the luminal contents and serves as a physical barrier against digestive enzymes, proliferating microorganisms, microbial components and byproducts, food-associated toxins, or other inflammatory stimuli[32]. In addition, it nurtures a thriving biofilm of microorganisms, the gut microbiome, which typifies the second mechanism of protection, the biological barrier[29,32]. Luminal microbiota are indispensable for the fermentation of indigestible carbohydrates, a process that provides the gut with short-chain fatty acids (SCFAs), mainly consisting of acetate,

propionate, and butyrate. Apart from being an important source of energy for host cells, SCFAs exhibit significant anti-inflammatory and anti-tumor effects and participate in various host signaling pathways, contributing to intestinal barrier integrity and metabolic homeostasis The commensal bacteria regulate choline bioavailability, promote the enterohepatic circulation of bile acids, and synthesize vitamin K and group B vitamins. In parallel, normal intestinal flora restrains the overgrowth of harmful microorganisms, a phenomenon referred to as colonization resistance, through nutrient antagonism[33]. Moreover, the microbiome-derived pathogen-associated molecular patterns (PAMPs) are recognized by pattern recognition receptors (PRRs) expressed by intestinal immune cells, indicating a relentless reciprocal dialogue between the microbiota and the intestinal immune system[6]. This leads to the third line of defense, that is, the immune barrier, which encompasses the gut-associated lymphoid tissue (GALT), effector and regulatory T cells (Tregs), immunoglobulin A (IgA)-secreting B (plasma) cells, innate lymphoid cells (ILCs), as well as macrophages and dendritic cells (DCs) of the lamina propria[5]. The cells of innate immunity carry an armamentarium of PRRs such as toll-like receptors (TLRs), nucleotide oligomerization domain-like receptors (NLRs), and retinoic acid-inducible gene-like receptors (RLRs) that recognize molecular pathogen-associated molecular patterns (PAMPs) or damaged-associated MPs (DAMPs) and orchestrate the well-tuned responses of the adaptive arm of the immune system.

The continuous crosstalk of the immune cells with the gut microbiome sustains the delicate balance between tolerance to beneficial bacteria and immunosurveillance against pathogenic species[6]. Commensal-derived signals and metabolites are recognized by myeloid cells in the lamina propria and orchestrate innate and adaptive immune responses[34]. Under homeostatic conditions, innate immune cells, such as macrophages and DCs, obtain a regulatory phenotype that promotes the secretion of anti-inflammatory molecules, *i.e.*, IL-10 and TGF-β. The production of IL-22 by type 3 ILCs supports tissue homeostasis and epithelial barrier integrity[35]. Properly-regulated antigen presenting cells (APCs) remain in the GALT or migrate to the mesenteric lymph nodes, where priming of naïve CD4+ T cells occurs, and stimulate Treg cell activity that plays a central role in the suppression of intestinal inflammation. In this immunomodulatory milieu, B cell activation and effective class-switching generate large numbers of IgA-secreting plasma cells that serve multiple functions, including protection against infection and maintenance of gut microbiome homeostasis[35].

THE INTESTINAL BARRIER FUNCTION IN COVID-19

SARS-CoV-2 disrupts the intestinal mechanical barrier

SARS-CoV-2 can invade and propagate in IECs by using the vastly expressed ACE2 and TMPRSS2 receptors on those cells[18,36]. In a mouse model, ACE2 was found to be a key inducer of intestinal stem cell proliferation and differentiation under pathologic conditions, while ACE2 deficiency was associated with a significant reduction of mucin-2 expression[37]. Accordingly, ACE2 knockout mice exhibited gut barrier dysfunction with subsequent leakage of bacterial components into the circulation[38]. Therefore, it could be hypothesized that interference of SARS-CoV-2 with ACE2 signaling could destabilize the mechanical barrier by interrupting the renewal of epithelial cells or by compromising mucus composition. Mucins create a protective matrix covering the epithelium and inhibit viral invasion, presumably *via* steric hindrance[39]. Even though evident macroscopic alterations were usually negligible, infection of the human small bowel by SARS-CoV-2 was associated with villi blunting and an aberrant accumulation of activated intraepithelial CD8+ T cells in the epithelium[36]. Cell trafficking could be driven by direct infection of IECs or could be the result of systemic immune activation due to COVID-19. In any case, the recruitment of intraepithelial CD8+ T cells enhanced the apoptotic process of IECs, as demonstrated by an upsurge in cleaved caspase-3+ apoptotic epithelial cells. This event was accompanied by a regenerative response of the epithelium marked by an increase in Ki67+ proliferating epithelial cells that extended beyond their typical localization in the crypts and occupied the villus compartment[36]. Evidently, SARS-CoV-2 can inflict a deleterious impact on the mechanical barrier through dysregulation of the balance between cell apoptosis and proliferation.

The function and integrity of epithelia and endothelia greatly depend on TJs, reflecting a putative mechanism of intestinal barrier injury in COVID-19 patients. The fundamental transmembrane proteins that frame TJs include the family of claudins (central regulators of paracellular permeability encompassing 26 members in humans), the junctional adhesion molecules, and the three junctional MARVEL domain proteins, *i.e.*, occludin, tricellulin, and MARVEL domain-containing protein 3. The most prominent intracellular junctional plaque components are zonula occludens (ZO) proteins (ZO1, ZO2, and ZO3), cingulin, and protein associated with LIN7 1 (PALS1)[40]. These proteins function as adaptor proteins or cytoskeletal linkers and participate in multiple extracellular and intracellular signaling pathways. Indeed, impairment of TJs occurs early in the course of COVID-19 and might represent the first hit in a multistage model of the disease[41]. SARS-CoV-2 infection decreases the expression of TJ proteins, such as occludin, claudin 5, and ZO-1 *in vivo*, and undermines the coherence of TJs between neighboring endothelial cells of the brain. As a result, TJs are haphazardly distributed, irregular, or gapped throughout the vascular endothelial layer, leading to derangement of the function

of the blood-brain barrier[42]. Cryo-electron microscopy and *in silico* modeling analyses have shown that SARS-CoV-2 uses its envelope (E) protein to interact with the TJ-associated PALS1 protein[43,44]. According to *in vitro* models of lung injury, the interplay between SARS E protein and PALS1 interrupts intracellular trafficking of E-cadherin, delays the formation of TJs and AJs, and affects epithelial polarity [45]. In a proof-of-concept study, Guo *et al*[46] developed a biomimetic human gut-on-chip model that reconstructs basic elements of the gut barrier, as it consists of IECs, endothelial, and mucin-producing cells under normal fluid flow and closely reproduces the pathophysiological processes of intestinal SARS-CoV-2 infection. Interestingly, IECs exhibit particularly high susceptibility to SARS-CoV-2 infection. Viral inoculation induces the dispersal of the physiological distribution of mucus-secreting cells and a profound reduction of both E-cadherin expression in the epithelium and VE-cadherin in the endothelium, which delineate serious impairment of AJs in the corresponding structures. As a consequence, widespread destruction of the villus-like complexes along with severe morphological remodeling of the vascular endothelium was observed[46]. IL-6 is a prominent mediator of inflammation and a reliable biomarker of disease severity in SARS-CoV-2 infection that was found to increase, in a sustained manner, endothelial permeability in a mouse model[47,48]. Administration of IL-6 induced vascular leakage and disruption of junctional localization of VE-cadherin and ZO-1 *via* Janus kinase-mediated signal transducer and activator of transcription 3 phosphorylation and *de novo* protein synthesis[48].

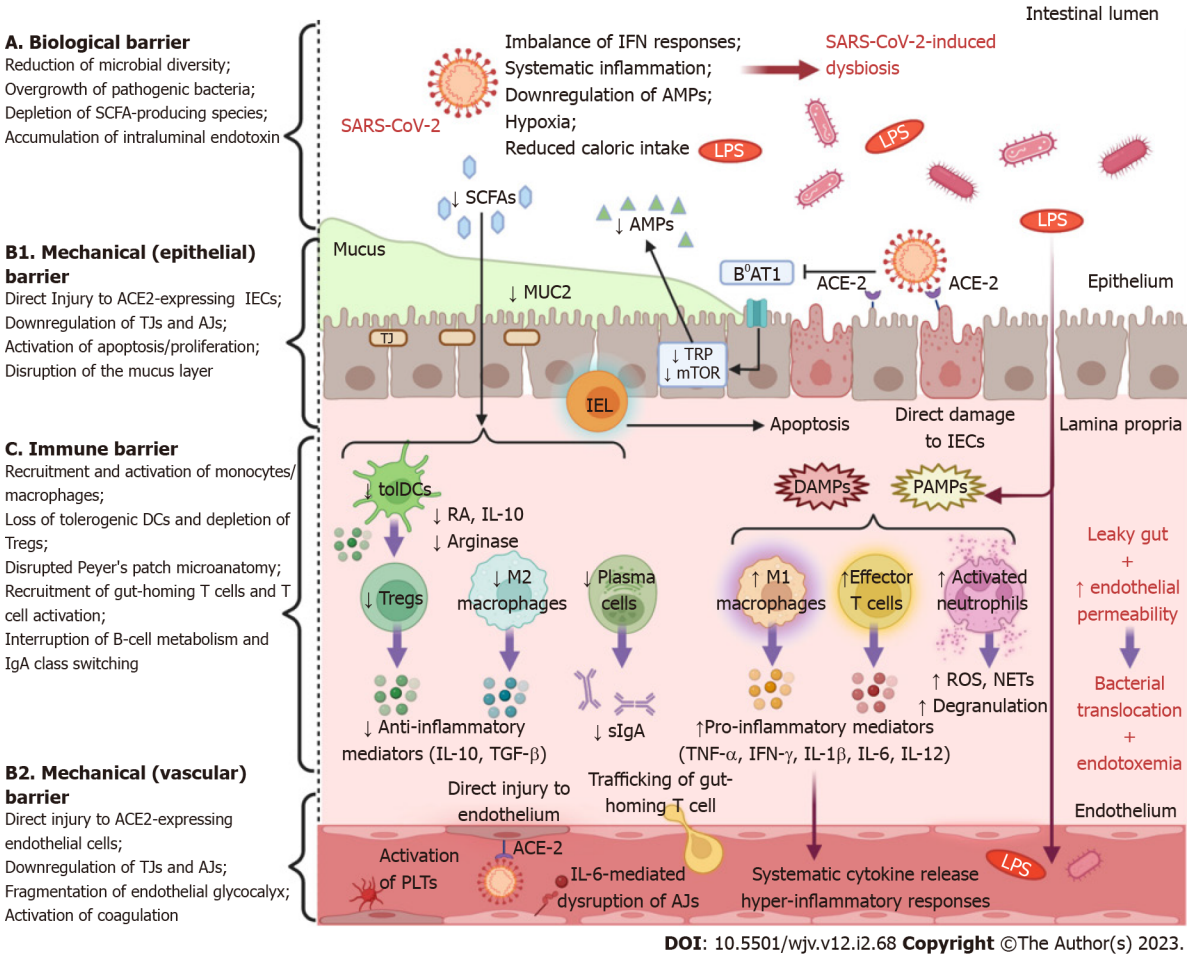
Serum levels of endotoxin and ZO-1 were significantly increased in patients with COVID-19-related pneumonia on admission compared to healthy controls. Importantly, endotoxemia is positively correlated with certain markers of inflammation, such as C-reactive protein (CRP) and ferritin[49]. Endotoxins are complex lipopolysaccharides (LPS), integral parts of the membrane of gram-negative bacteria, and potent drivers of inflammation. Indeed, endotoxemia can occur as a result of gram-negative bacteremia; however, endotoxemia is most commonly caused by a compromised gut barrier [50]. ZO-1 is a peripheral membrane scaffolding protein and a basic constituent of TJs that fulfills versatile functions including establishment of cell-cell adhesion, modulation of the paracellular barrier, regulation of cell migration and angiogenesis, and induction of mucosal repair processes[51,52]. Previous studies have confirmed that serum ZO-1 represents a reliable biomarker of disrupted paracellular permeability, as it inversely correlates with intestinal ZO-1 expression in diverse pathologic conditions[53,54]. These results are consistent with another study, in which severe SARS-CoV-2 infection presented key features of gut barrier dysfunction *in tandem* with increased intestinal permeability. Specifically, patients with severe COVID-19 presented with endotoxemia and higher serum levels of zonulin, occludin, and regenerating family member 3 alpha, indicating severe impairment of the intestinal epithelial barrier[55].

SARS-CoV-2-induced gut microbiome dysbiosis

In the course of evolution, hosts and their microbial ecosystems have mutually developed, forging an intimate relationship of interdependence. Dysbiosis refers to alterations in the composition, quantity, or distribution of the gut microbiome. This condition is characterized by a predominance of pathogenic species and has been linked to the pathophysiology of numerous intestinal and extraintestinal disorders [56]. There is a growing body of data suggesting that COVID-19 is associated with drastic alterations of the normal intestinal flora, even when removing the confounding effect of antibiotics (Figure 1)[57-59]. In addition, SARS-CoV-2-induced dysbiosis appears to persist long after the resolution of symptoms and might be a predisposing factor for long-term complications in COVID-19 patients[57,58,60].

The mechanisms underlying COVID-19-related dysbiosis have not yet been fully elucidated. The interactions between SARS-CoV-2 and the ACE2 receptor can influence the composition of gut microbiota by interfering with the secretion of AMPs. The activity of the amino acid transporter B^0AT1, which mediates the intestinal uptake of tryptophan, depends on ACE2 signaling[61]. Tryptophan regulates AMP production through the mammalian target of rapamycin pathway[62]. Therefore, tryptophan depletion due to ACE2 blockade can interrupt AMP production, and thus, perturb the intraluminal microbial community. Commensal bacteria, in turn, contribute to mucosal homeostasis by regulating ACE2 expression in the gut[63]. The release of pro-inflammatory cytokines, particularly TNF-α, during respiratory infections has a powerful anorexigenic effect *via* hypothalamic action. The ensuing reduction in caloric intake and dietary fibers disturbs the composition of the gut microbiota and the production of their metabolites. In animal models of respiratory syncytial virus (RSV) and influenza infection, the neutralization of anorexigenic cytokines prevented weight loss and mitigated gut microbiome alterations[64]. Alternatively, SARS-CoV-2 infection can cause dysbiosis through an imbalance of systemic or intestinal IFNs[65]. In this regard, in an influenza mouse model, the microbiome was amenable to significant changes *via* an IFN-type I-dependent mechanism[66]. Hypoxia is a serious feature of severe COVID-19 and hypoxic stress could be an important instigator of dysbiosis by dysregulation of hypoxia-inducible factor signaling[67]. Finally, local epithelial injury, which results in leaky gut and DAMP secretion, might disrupt the immune control of microbial homeostasis and could further aggravate the dysbiotic state[68].

In their recently published meta-analysis, Farsi *et al*[69] offered a thorough synthesis of the gut microbiota changes in COVID-19 patients. At the phylum level, dysbiosis is typified by a decrease in the *Firmicutes* to *Bacteroidetes* ratio. More specifically, COVID-19 is associated with a decrease in important



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Figure 1 Schematic representation of intestinal barrier dysfunction in severe acute respiratory syndrome coronavirus 2 infection. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is associated with a multifaceted dysfunction of the gut barrier as it exerts detrimental effects on all of its major levels of defense. A: Systemic inflammation, an imbalance of interferon (IFN) responses, hypoxia or low blood flow, and reduced caloric intake, due to coronavirus disease 2019-associated cachexia, contribute to intestinal microbiome alterations. Gut dysbiosis is characterized by an overgrowth of opportunistic pathogens, which are a source of harmful substances, *e.g.*, endotoxin, peptidoglycan, β-glucan, as well as a depletion of commensal microorganisms, which synthesize beneficial metabolites such as short-chain fatty acids; B: SARS-CoV-2 induces either direct cytopathic injury to angiotensin-converting enzyme 2 (ACE2)-expressing intestinal epithelial cells (IECs) and endothelial cells, or indirect immune-mediated damage, or both. Moreover, the secretion of mucin glycoproteins, the fundamental element of the mucus layer, is severely impaired. In addition, the virus undermines the expression of tight junction and adherens junction proteins, leading to increased paracellular permeability and structural destabilization of the epithelium and endothelium. Viral interference with ACE2 signaling attenuates the activity of the amino acid transporter B0AT1, which is necessary for intestinal absorption of tryptophan (TRP). As a result, TRP depletion downregulates the mammalian target of rapamycin pathway, which promotes the expression of antimicrobial peptides. The overwhelming intraepithelial CD8+ lymphocytes trigger the apoptosis of IECs, which is followed by reactive regeneration. In parallel, disease-activated and apoptotic endothelial cells are potent drivers of neutrophil/monocyte recruitment, platelet aggregation, and coagulation activation; C: Commensal-derived metabolites modulate innate immune responses by inducing tolerogenic dendritic cells and M2-polarized macrophages. SARS-CoV-2-induced dysbiosis eliminates these immunoregulatory effects, inhibits T regulatory (Treg) cell activity, and suppresses the secretion of anti-inflammatory cytokines such as interleukin 10 (IL-10) and transforming growth factor beta. The release of damage-associated molecular patterns from injured cells and the influx of pathogen-associated molecular patterns, as a result of leaky gut, orchestrate the recruitment and activation of innate immune cells that secrete pro-inflammatory mediators (IFN-γ, tumor necrosis factor alpha, IL-1β, IL-6, IL-12). Subsequently, this pro-inflammatory microenvironment precipitates the derangement of adaptive immune responses, as demonstrated by increased trafficking of gut-homing T cells and effector T cell predominance. Furthermore, interruption of B-cell metabolism, plasma cell differentiation, and immunoglobulin A (IgA) class switching impede the effective secretion of protective dimeric IgA. This multi-layered disruption of intestinal barrier integrity allows the dissemination of intraluminal bacteria and endotoxin into the mesenteric lymphatic vessel or/and into the systemic circulation (created with biorender.com). IEL: Intraepithelial lymphocyte; LPS: Lipopolysaccharides; MUC2: Mucin 2; NETs: Neutrophil extracellular traps; PLTs: Platelets; RA: Retinoic acid; ROS: Reactive oxygen species; sIgA: Secretory immunoglobulin A; SCFAs: Short-chain fatty acids.

butyrate-producing bacteria, including *Faecalibacterium* and *Roseburia*[69-71]. The genus *Roseburia* contributes to mucosal integrity and colonic motility and exerts significant anti-inflammatory effects by modulating IL-10 production[72]. Similarly, *Faecalibacterium prausnitzii* is a valuable gut symbiont with recognized anti-inflammatory effects in IBD patients *via* inhibition of the nuclear factor kappa B pathway[73]. The gut microbiome of COVID-19 patients is also depleted of several other beneficial genera such as *Alistipes*, *Eubacterium*, and *Bifidobacterium*[69]. The genus *Eubacterium* consists of a phylogenetically diverse group of SCFA-producing bacteria that contribute to bile acid metabolism and exhibit compelling associations with intestinal health[74]. *Bifidobacterium* strains prompt Treg responses, induce tolerogenic DC phenotypes, and demonstrate vigorous antimicrobial and antiviral activity[75].

Of note, their ability to suppress gut dysbiosis and promote mucosal homeostasis has paved the way for researchers to investigate the therapeutic efficacy of *Bifidobacterium*-based probiotic preparations in several diseases, including irritable bowel syndrome, inflammatory bowel disease (IBD), or COVID-19 [76-78]. On the other hand, the COVID-19-derived gut microbiome is relatively enriched in opportunistic pathogens such as *Streptococcus*, *Bacteroides*, *Rothia*, *Veillonella*, *Actinomyces*, and *Eggerthella* [69,70]. Although the genus *Ruminococcus* is generally underrepresented, certain species such as *Ruminococcus gnavus* and *Ruminococcus torques* are significantly more abundant in COVID-19 patients. This is not surprising considering that *Ruminococcus gnavus* and *Ruminococcus torques* are harmful bacteria that degrade mucin glycans to harvest energy, secrete pro-inflammatory mediators, and are involved in IBD pathogenesis[79,80].

The degree of microbiome alterations correlates well with COVID-19 severity, and identification of early patterns of dysbiosis could lead to a microbiome-based stratification of patients according to their risk of progressing to severe COVID-19[81]. Indeed, the commensal genera *Faecalibacterium* and *Roseburia* are depleted in the gut microbiome of patients with critical disease[57,82]. The baseline abundance of *Clostridium ramosum* and *Clostridium hathewayi* is also associated with COVID-19 severity and could represent risk factors for portal vein thrombosis[69,82]. The genus *Enterococcus* is overrepresented in COVID-19 patients who necessitate ICU admission or developed bloodstream infections, whereas, surprisingly, the gut microbiome of other critically ill non-COVID-19 patients is devoid of this bacterium[83]. The reduction of the *Firmicutes*/*Bacteroidetes* ratio is indicative of severe disease, which is mainly attributed to the depletion of fiber-utilizing bacteria, namely *Faecalibacterium prausnitzii*, *Phocaeicola plebeius,* and *Prevotella*[84]. These findings are consistent with an interesting study exploring the role of gut microbiota as predictors of disease severity[81]. A lower *Firmicutes*/*Bacteroidetes* ratio, a higher prevalence of *Proteobacteria,* and an exhaustion of commensal butyrate-producing microorganisms are more evident in severely ill patients, while a lower bacterial diversity, defined by the Shannon diversity index, is identified as a prognostic biomarker of disease severity[81]. In agreement with this, the fecal microbiome of patients requiring mechanical ventilation has demonstrated low bacterial richness as assessed by Shannon or Chao1 indices[81,85,86]. Implementation of a multiomics approach to decipher the dysregulation of metabolic and microbial signatures during COVID-19 could provide a basis for the development of novel microbiome-targeted therapeutics[87].

SARS-CoV-2 deranging mucosal immune system responses

The mucosal immune system is the largest immunologically aware organ in the body, committed to maintaining the equilibrium between active protection against pathogens and immune-tolerance to commensal microorganisms, dietary substances, and self-antigens. The gut-derived metabolites orchestrate immune cell responses and differentiation and impart a critical role in mucosal homeostasis [56,88]. In particular, the transcriptional "education" of innate immune cells is strongly influenced by intestinal microbiota metabolites[56]. Butyrate, for example, induces M2-like polarization of macrophages, which shapes an immunomodulatory milieu by increasing the expression of arginase 1 and suppressing TNF-α, IL-6, IL-12b, and nitric oxide synthase 2[89]. As regards adaptive immunity, butyrate enhances transcription of the forkhead box P3 gene in naïve T cells through inhibition of histone deacetylases, and thereby, expands Treg cell populations[90]. The capsular polysaccharide A, which originates from the prominent human symbiont *fragilis*, promotes the proliferation of Treg cells in the lamina propria and shapes a pro-inflammatory microenvironment rich in IL-10[91]. Vitamin A or RA, produced by the gut commensal *Bifidobacterium infantis*, enhances the expression of aldehyde dehydrogenase 1 family member A2 encoding retinal dehydrogenase 2 in resident DCs of the mucosa. Subsequently, gut-modulated DCs secrete high levels of RA that drive naïve T cell differentiation into Treg cells[92]. Treg cell generation in the intestinal mucosa is also triggered by β-glucan polysaccharides deriving from the cell surface of *Bifidobacterium bifidum*[93]. In addition, B cell metabolism and differentiation in mucosal and systemic tissues are regulated by gut-derived SCFAs, emphasizing the significance of symbionts in effective antibody production[94]. Apparently, the depletion of SCFA-producing microorganisms or other beneficial species as well as gut metabolome modifications might have a detrimental impact on mucosal immunity in the course of COVID-19.

Defensins are prominent members of the AMP family with multifaceted immunomodulatory functions and broad antimicrobial and antiviral activity. Defensins provide protection against SARS-CoV-2 infection not only by maintaining gut microbiome homeostasis but also by inhibiting viral fusion *via* interference with ACE2 receptors[95,96]. Dysregulation in the expression of various defensin genes was evident following infection with COVID-19[97]. IFNs are multipotent cytokines of innate immunity with crucial role in the containment of viral infections. The proliferation of SARS-CoV-2 in human gut cells is effectively inhibited by type I (IFN-α, IFN-β) and type III IFNs (IFN-λs); however, type III IFNs elicit a more profound and long-lasting antiviral effect[98,99]. In order to escape immune surveillance, SARS-CoV-2 has developed strategies to proliferate stealthily into cells without eliciting strong IFN responses[65]. Alternatively, SARS-CoV-2 can interfere with IFN-γ (type II IFN) signaling to boost its infectively in the gut. In human colonic organoids, IFN-γ drives cellular differentiation towards ACE2-expessing epithelial cells, which are highly susceptible to SARS-CoV-2 infection[100]. Moreover, IFN-γ acts synergistically with TNF-α to instigate inflammatory cell death and tissue damage[101].

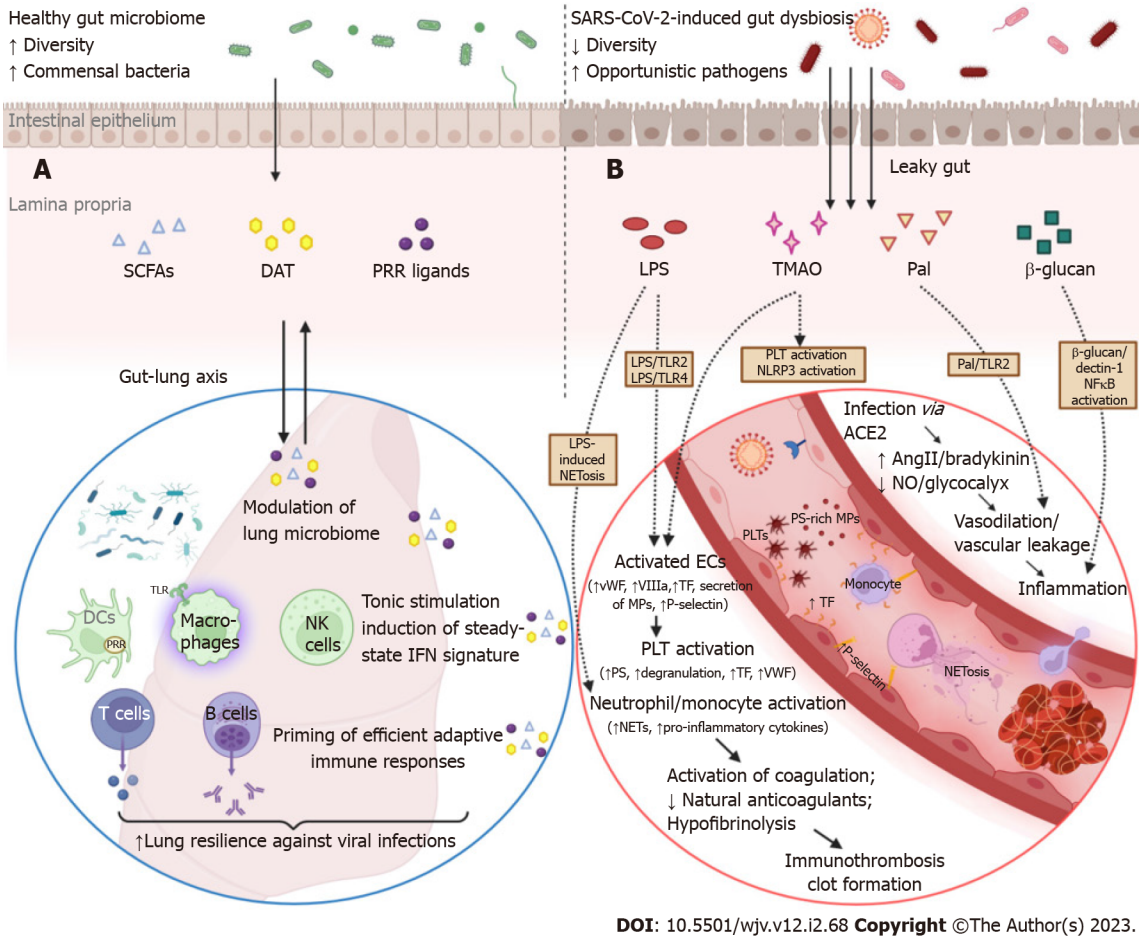
Elevated levels of plasma markers of inflammation and immune activation are hallmarks of severe COVID-19. Similarly, the expression of genes encoding pro-inflammatory mediators, including TNF-α, IL-6, chemokine (C-C motif) ligand 5 (CCL5), chemokine (C-X-C motif) ligand 1 (CXCL1), CXCL10, CXCL11, and CSF-3 were upregulated in digestive tissues in both *in vitro* and *in vivo* models of SARS-CoV-2 infection[46,102]. Compared to uninfected individuals, patients with COVID-19 have increased fecal levels of pro-inflammatory cytokines such as IL-8 and IL-18, whereas IL-23 is higher in patients with severe disease[103,104]. Strikingly, analysis of human ileal biopsies has demonstrated that several pro-inflammatory genes including *IFNG*, *CXCL8*, *CXCL2*, and *IL1B* are downregulated. This pro-inflammatory response is associated with milder symptoms and improved outcomes, revealing an immunomodulatory function of the GI in controlling SARS-CoV-2 infection[18]. Nevertheless, molecular events in severe COVID-19, due to increased bacterial translocation and systemic immune activation, might eliminate the immunoregulatory functions of the GI. Indeed, post-mortem evaluation of COVID-19 patients' intestinal tissues revealed the overexpression of TNF-α and IL-10, which exerts antithetical effects[105]. The presence of IL-10 in the gut and lung may have immunosuppressive effects by inhibiting the expression of the human leukocyte antigen DR isotype (HLA-DR) on APCs[105]. Depletion of HLA-DR expression on monocytes is a major characteristic of immune dysregulation in COVID-19 patients who develop severe respiratory failure[106].

Essentially, changes in the expression of cytokines and downstream dysregulation of their signaling networks mirror alterations in the composition of immune cell populations of the lamina propria. Imaging mass cytometric analysis showed that the intestinal tract of deceased patients with COVID-19 accommodated higher numbers of CD11b+ macrophages, CD11c+ DCs, natural killer T cells, and B cells compared to healthy controls[105]. Another study, which evaluated post-mortem tissues of COVID-19 patients, demonstrated severe disorganization of ileal Peyer's patches with loss of B cell/T cell zonation and depletion of the germinal center. In addition, impaired interactions between B and T cells, an enhanced number of follicular macrophages, the reduction of CD27+ memory B cells, and downregulation of CD74 expression on B cells were reported[107]. Livanos *et al*[18] in their study, which supports an anti-inflammatory function of the GI in SARS-CoV-2 infection, showed that the lamina propria of COVID-19 patients was depleted of conventional DCs and plasmacytoid DCs, whereas effector CD4+ and CD8+ T cells as well as tissue resident memory T cells were increased. Lehmann *et al*[36]. revealed that the activation and proliferation of antigen-experienced intraepithelial CD8+ T cells into the intestinal mucosa was associated with epithelial barrier dysfunction in COVID-19 patients, while monocytes and macrophages of the duodenal mucosa expressed markers indicative of their recent recruitment from the circulation. In agreement, levels of CCL25, a gut homing marker, were increased in the sera of COVID-19 patients, suggesting that intestinal inflammation might result from CCL5/CCR9-mediated trafficking of gut-specific T cells into the mucosa[108,109]. Humoral immune responses are vital for counterattacking viral invasion through the production of neutralizing antibodies. Secretory dimeric IgA is the predominant mucosal antibody and an integral component of the immunological barrier[6]. IgA antibody overproduction dominates the early pre-specific humoral response to SARS-CoV-2 infection, while virus-specific IgA antibodies display more robust neutralizing capacity compared to their monomeric IgG counterparts[110]. Interestingly, the levels of mucosal SARS-CoV-2-specific IgA are inversely correlated with age[111]. Therefore, it can be inferred that the inability of B cells to mount an effective IgA response contributes to excessive viral propagation in the course of severe COVID-19[112].

THE GUT-LUNG AXIS IN COVID-19

As mentioned above, homeostasis of the gut microbiome can be immensely affected in the course of respiratory tract infections such as COVID-19. Conversely, intestinal microbiota plays an important role in fine-tuning the systemic immune system and eliciting efficient antiviral responses to address lung infections[113-115]. Gut bacterial components and metabolites can enter portal circulation or mesenteric lymphatics, which drain to the cisterna chyli first, then to the thoracic duct, and finally to the left subclavian vein. Intriguingly, the pulmonary vascular bed is the first to interact with the mesenteric lymph, implicating the importance of gut-derived signals in shaping lung immune responses in health as well as in driving ARDS in critically ill patients[116]. Under normal conditions, commensal-associated stimuli provide an indispensable for optimal antiviral activity, tonic activation of the host's innate immunity through their impact on alveolar macrophages, resident DCs, and lung epithelial cells (Figure 2)[117]. In contrast, germ-free mice are unable to evoke strong innate and adaptive immune responses and, thus, experience feeble control of viral infections and unfavorable outcomes[117,118]. Although the intestinal and respiratory tracts are anatomically distinct compartments, their mucosal immune cells and microbial communities configure a bidirectional "gut-lung" axis cross-talk that is highly pertinent to COVID-19 pathogenesis.

The mechanisms through which intestinal flora reinforces lung resilience against viral invasion have only recently begun to be unraveled with the assistance of murine models. The gut microbiota orchestrates the steady-state IFN signature in lung stromal cells, which protects against early influenza virus



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Figure 2 Overview of the sequelae of gut barrier dysfunction in severe coronavirus disease 2019: dysregulation of lung immune responses and establishment of a prothrombotic state. A: Although the intestinal and respiratory tracts are anatomically distinct compartments, their mucosal immune cells and microbial ecosystems participate in a bidirectional immunological crosstalk (gut-lung axis). An intact intestinal barrier is pivotal in maintaining lung microbiome homeostasis and fine-tuning the respiratory immune system to elicit potent antiviral responses in the case of infection. Commensal bacteria provide tonic stimulation (through the production of pattern-recognition receptor-ligands, desaminotyrosine, short-chain fatty acids, *etc.*) of the epithelial, stromal, and innate immune cells of the lungs and modulate the steady-state interferon-signature, which is essential for suppressing the early phase of viral proliferation. In addition, gut-derived signals and metabolites orchestrate the effective priming of adaptive immune responses by inducing the differentiation of virus-specific CD4+ and CD8+ T cells and antibody-secreting plasma cells, which are responsible for viral control and clearance in the later stages of infection. In coronavirus disease 2019 (COVID-19), gut barrier dysfunction and depletion of symbiotic microorganisms eliminate the aforementioned immunomodulatory effects and compromise the ability of the respiratory immune system to effectively contain severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection; B: The gut vascular bed has a massive endothelial surface that is susceptible to SARS-CoV-2 infection. SARS-CoV-2 can inflict direct injury to angiotensin-converting enzyme 2 (ACE2)-expressing endothelial cells; interruption of ACE2 signaling can dysregulate both the renin-angiotensin and kinin-kallikrein systems, leading to vascular leakage. The activated or apoptotic endothelial cells release phosphatidylserine-rich endothelial microparticles and secrete large amounts of tissue factor, VIIIa, von Willebrand factor, and other procoagulant cofactors. Circulating platelets accumulate at sites of vascular injury, adhere to each other, and become activated, leading to further secretion of prothrombotic substances. Overexpression of adhesion molecules, such as P-selectin, facilitates the recruitment and activation of monocytes and other leukocytes, including neutrophil extracellular trap (NET)-producing neutrophils. COVID-19-associated immunothrombosis refers to this concurrent aberrant activation of the innate immune and coagulation systems, which predisposes to serious thrombotic complications. This vicious cycle can be further exacerbated by gut barrier dysfunction. Low-grade endotoxemia, due to increased intestinal permeability, enhances the activation of endothelial cells and platelets by inducing lipopolysaccharide (LPS)/toll-like receptor 2 (TLR2) and LPS/TRL4 downstream signaling pathways. In parallel, LPS is a potent driver of NET formation. Several other bacterial lipoproteins, such as peptidoglycan-associated lipoprotein (Pal) or Pam^3Cys, aggravate vascular leakage and precipitate thrombus formation through TLR2 activation. Moreover, translocation of fungal components, such as β-glucan, could directly stimulate leukocytes and promote inflammation by binding to the Dectin-1 receptor and activating the nuclear factor-κB pathway. Finally, gut dysbiosis is associated with trimethylamine N-oxide overproduction, which is a recognized risk factor for clotting events as it enhances platelet hyperresponsiveness, endothelial dysfunction, and NLR family pyrin domain containing 3 inflammasome activation (created with biorender.com). AngII: Angiotensin II; DCs: Dendritic cells; ECs: Endothelial cells; NK cells: Natural killer cells; NO: Nitric oxide; PLT: Platelet; DAT: Desaminotyrosine; SCFAs: Short-chain fatty acids; PRRs: Pattern recognition receptors.

proliferation by driving the expression of IFN-α/β receptor[113]. In line with this, when mice were exposed to antibiotics, their macrophage-related type I and type II IFN responses were severely impaired, resulting in the uncontrolled spread of systemic lymphocytic choriomeningitis virus or mucosal (influenza virus) infection. In a murine model of influenza infection, desaminotyrosine (DAT), a degradation product of dietary flavonoids, induced an efficient amplification of the IFN type I loop, which suppressed viral gene expression and airway epithelial damage. Notably, a distinct human-

derived commensal, *Clostridium orbiscindens*, produced DAT and rescued antibiotically-treated mice from viral infection[119]. An outer membrane glycolipid originating from the gut symbiont *Bacteroides fragilis* protected mice against viral infection through the induction and systemic release of IFN-β by colonic DCs[120]. Acetate, another metabolite derived from the gut microbiome, is able to enter the circulation, enhance IFN-β signal transduction *via* stimulation of the G-protein-coupled receptor 43 in pulmonary epithelial cells, and thus limit severe RSV infection[114]. Moreover, depletion of commensal bacteria precipitated significant epigenetic modifications at the level of mononuclear phagocytes residing in non-mucosal lymphoid organs. As a result, these cells were unable to induce type I IFN secretion, which led to suboptimal NK cell priming and poor antiviral responses[121]. Admittedly, an imbalance of IFN signaling is an inherent characteristic of the pathogenesis of respiratory tract infections, including SARS-CoV-2-associated pneumonia[122]. Early severe COVID-19 is governed by overwhelming IFN-α responses as well as NK cell functional exhaustion, which is manifested by abnormal expression of interferon-stimulated genes[123]. Therefore, it is plausible to assume that metabolites and signals stemming from luminal bacteria could influence the early response to SARS-CoV-2 infection by altering the IFN signature and compromising innate immunocompetence in the lungs.

Regulation of the mucosal immune system of the respiratory tract, which supervises airway colonization, depends on reciprocal signaling with the gut. The respiratory tract is not sterile; instead, it harbors a unique microbial ecosystem and its role in homeostasis and disease is being increasingly recognized with the advent of culture-independent molecular techniques[124]. Alterations of the lung microbiome signature are associated with clinical outcomes in critically ill patients infected with SARS-CoV-2[125]. The immunomodulatory potential of airway bacteria was exemplified by a pathogen-free murine model of influenza virus. Colonization of the upper respiratory tract by *Staphylococcus aureus* induces the recruitment of peripheral macrophages into the alveoli and their polarization toward an M2-like phenotype *via* TLR2 signaling. As a result, *Staphylococcus aureus* creates an anti-inflammatory pulmonary milieu that attenuated immune-mediated injury and prevented lethal influenza infection [126].

The robust and timely priming of adaptive immunity is necessary to contain SAR-CoV-2 infection. Delayed activation of adaptive immune responses and depletion of virus-specific T cells are hallmarks of severe or fatal COVID-19[127]. Interestingly, multiple studies have revealed that the gut microbiome is actively involved in shaping adaptive immunity in the respiratory tract. In a murine model of West Nile virus infection, exposure to antibiotics impaired the ability to elicit potent T cell responses, decreased the number of virus-specific CD8+ T cells, and led to worse disease outcomes[118]. Ichinohe *et al*[115] demonstrated that intestinal microflora regulates an even broader spectrum of adaptive immunity responses in the respiratory mucosa. More specifically, neomycin-sensitive commensal bacteria were essential for the induction of effective humoral responses and the generation of virus-specific CD4+ and CD8+ T cells in the lungs. Normal intestinal flora provided signals that maintained a steady-state IL-1β, pro-IL-18, and NLR family pyrin domain containing 3 (NLRP3) expression. Antibiotic-induced immunodeficiency is attributed to impaired inflammasome activation, abnormal activation and distribution of respiratory DCs, and inadequate DC migration to draining lymph nodes of the lung[115]. In this setting, Gauguet *et al*[128] demonstrated that the presence of segmented filamentous bacteria in the murine microbiome was vital for priming strong Th17 immunity responses and IL-22 secretion in the respiratory system. In response to fiber supplementation, the gut microbiome generates large amounts of SCFAs, which hinders influenza-induced lung injury through a dual mechanism concerning both arms of immunity[129]. First, SCFAs enhances the cellular metabolism and the effector functions of CD8+ T cells in the respiratory tract. Second, SCFAs induce an alternative activation of macrophages, which exhibit the limited ability to express the chemokine CXCL1. As a result, SCFA-modulated macrophages reduce early neutrophil infiltration and subsequent injury in the airways[129]. In a randomized controlled trial, non-hospitalized patients with symptomatic COVID-19 were allocated 1:1 to groups receiving an oral probiotic formulation or placebo for 30 d. Remarkably, probiotic supplementation was associated with higher rates of complete remission, decreased nasopharyngeal viral levels, and shorter duration of symptoms by inducing vigorous virus-specific IgM and IgG antibody responses.

In summary, a well-preserved intestinal barrier function, harboring a diverse consortium of commensal bacteria, provides the essential signals for appropriate and effective modulation of immune system responses in the lungs. Regardless of being the cause or the result of severe SARS-CoV-2 infection, gut dysbiosis is not a silent bystander but an active orchestrator of dysregulated immune responses in the respiratory tract. Modulation of gut microbiota represents an emerging therapeutic intervention to mitigate immune-mediated lung injury and improve COVID-19 outcomes[130].

GUT BARRIER DYSFUNCTION INFLAMING COVID-19-ASSOCIATED COAGULOPATHY

COVID-19-associated coagulopathy (CAC) is a life-threatening condition that can lead to arterial thromboembolism (ATE), such as acute coronary syndrome and cerebrovascular accident, or venous

thromboembolism (VTE), which manifests as deep vein thrombosis or pulmonary embolism[131]. Mild thrombocytopenia, prolonged prothrombin time, and increased serum levels of fibrinogen, CRP, P-selectin, and d-dimers have all been linked to clotting events in COVID-19 patients; these deviations become more pronounced as the disease progresses[131,132]. A recent meta-analysis, comprised of more than 90000 patients, concluded that hospitalized COVID-19 patients have a significantly increased 90-d risk of VTE in comparison to hospitalized individuals with influenza virus (9.5% *vs* 5.3%), and that this peril persists even after the breakthrough of effective vaccines[133]. COVID-19-related thromboembolic events are major burdens of morbidity and mortality, and their incidence increases with disease severity, affecting up to one third of patients in ICUs[134-136]. The pathophysiology underlying CAC is multifactorial, encompassing endothelial injury, over-exuberant immune responses, and overt dysregulation of coagulation and fibrinolytic pathways, which collectively result in a procoagulant state[131]. The activation of these mechanisms can be further aggravated by defects in intestinal barrier integrity due to SARS-CoV-2 infection[27,137].

The dysfunction of the endothelium is a hallmark of COVID-19, representing a common feature in multiple clinical manifestations of the disease such as thromboembolic events, neurological complications, and renal dysfunction[138,139]. The intestinal tract accommodates an enormous vascular endothelial surface consisting of a monolayer of squamous endothelial cells. Under physiological conditions, endothelial cells regulate the vascular tone and secrete anticoagulant and antiplatelet agents that preclude clotting events[140]. Human endothelial cells express the key cofactors, namely ACE2 and TMPRSS2, which are exploited by SARS-CoV-2 to invade its target cells[141]. There is evidence of viral inclusions in endothelial cells and mononuclear cell infiltrates in the walls of small vessels, as well as markers of endothelial cell apoptosis[142]. SARS-CoV-2-induced dysfunction of gut microvasculature and fragmentation of the endothelial glycocalyx eliminate these protective effects, promote vasoconstriction due to depletion of endothelium-derived nitric oxide, and drive platelet activation and fibrin formation[131]. Indeed, mesenteric thrombosis is not uncommon in critically ill patients with COVID-19, while focal ischemic lesions, inflammation of the endothelium, vessel wall edema, microhemorrhage, and microthrombi are frequent findings in resected bowel segments[27,143-145]. SARS-CoV-2-mediated dysregulation of ACE2 signaling in intestinal vascular endothelium might create a prothrombotic microenvironment through a dual mechanism. First, ACE2 catalyzes the conversion of angiotensin (Ang II) to angiotensin 1-7 (Ang 1-7), which confers important antithrombotic and immunoregulatory effects by binding to G-protein coupled Mas receptors. Therefore, ACE2 depletion attenuates the Ang 1-7 downstream pathway and shifts the balance in favor of Ang II, which binds to its cognate receptor and exerts harmful prothrombotic and inflammatory effects[146]. Second, the kinin-kallikrein system is also directly modulated by ACE and ACE2. Thus, the ACE/ACE2 imbalance can induce a "kinin storm" and amplify vascular permeability, cell migration, platelet activation, and oxidative stress[131,147].

There are data supporting the hypothesis that SARS-CoV-2 inflicts damage on endothelial cells (endotheliitis) *via* both direct cytopathic and indirect immune-mediated mechanisms[148]. Activated or apoptotic intestinal endothelial cells retract their margins, release endothelial microparticles (MPs), and abolish their ability to confine phosphatidylserine (PS) into the inner layer of the cellular membrane[27,149]. In this setting, microRNA expression in endothelial-derived extracellular vesicles has been associated with cerebrovascular events in COVID-19 patients by compromising the function of the blood-brain barrier[150,151]. Endothelial MPs and PS-positive filopods support the formation of the prothrombinase complex along with activated coagulation factors Va and Xa and thereby catalyze a pivotal step of the coagulation cascade, that is, the proteolytic activation of thrombin[27,152]. In parallel, exposure of subendothelial tissues and tissue factor (TF) to plasma procoagulants triggers the extrinsic coagulation pathway[132]. Damaged endothelial cells sustain this hypercoagulable milieu and promote platelet aggregation by secreting large amounts of factor VIII and von Willebrand factor (VWF), respectively[153-156]. Thrombotic microangiopathy is further exacerbated due to relative deficiency in metalloproteinase ADAM metallopeptidase with thrombospondin type 1 motif 13 activity in COVID-19 patients, which leads to insufficient VWF cleavage and enhanced platelet-vessel wall interactions[157]. Numerous other endothelial dysfunction markers, such as circulating endothelial cells, soluble (s)E-selectin, soluble thrombomodulin, and soluble intercellular adhesion molecule 1 are significantly increased in COVID-19 patients receiving ICU care[153,155,158]. Furthermore, critically ill patients demonstrate functional exhaustion of natural anticoagulants (protein C, protein S, and antithrombin) and develop anti-phospholipid antibodies at high rates[154]. This procoagulant state is accompanied by suboptimal fibrinolytic potential and remodeling of the clot structure, which displays a denser fibrin network as well as thinner and shorter fibrin fibers. In severe COVID-19, hypofibrinolysis is dictated by an upregulation of plasminogen activator inhibitor-1 and its stabilizing cofactor vitronectin, which reduce plasminogen generation, despite concurrent elevations of tissue plasminogen activator[159].

Immunothrombosis illustrates the intricate cross-talk between the innate immune system and the coagulation pathway, which aims to locally contain an infection by facilitating recognition and eradication of invading pathogens. An aberrant activation of immunothrombosis is associated with severe thrombotic complications in SARS-CoV-2 infection[160]. Endothelium expresses a variety of adhesion and chemoattractant molecules, promoting the recruitment of monocytes and neutrophils at sites of injury. The release of DAMPs and PAMPs stimulates innate immune cells, which subsequently express TF and pro-inflammatory mediators in large amounts and precipitates the formation of

neutrophil extracellular traps (NETs)[156]. NETs are web-like structures consisting of DNA complexed with histones, bactericidal enzymes, complement factors, and coagulants. NETosis is a potent driver of immunothrombosis in COVID-19 and contributes to the procoagulant state in IBD through various mechanisms: cleavage of natural anticoagulants, direct activation of the contact-dependent and extrinsic pathways of coagulation, and aggregation of platelets[156,161]. In turn, activated platelets overexpress P-selectin and other adhesion molecules, which enhance their interactions with monocytes and NETs [162]. Through the secretion of their intracellular prothrombotic granule substances and externalization of their PS-rich membrane, platelets trigger and sustain thromboinflammation[131].

There is evidence that this vicious cycle could be further aggravated by gut barrier dysfunction. In particular, Oliva *et al*[163] reported that serum LPS and zonulin were increased in hospitalized COVID-19 patients and showed that endotoxemia was an independent predictor of in-hospital thrombotic complications. Zonulin is involved in the modulation of gut permeability by orchestrating the disassembly of intercellular TJ[53]. In this regard, serum LPS positively correlated with zonulin, supporting that low-grade endotoxemia precipitating thrombosis in COVID-19 originates from the gut [163]. In addition, thrombogenesis in patients with endotoxemia was associated with the TLR4-dependent activation of platelets[163]. In agreement, LPS/TLR4 and LPS/TLR2 downstream signaling pathways have been previously implicated in procoagulant conditions by promoting endothelial cell and platelet activation[164]. Several other bacterial lipoproteins, such as Pam^3Cys and peptidoglycan-associated lipoprotein, promote vascular leakage and thrombus formation through TLR2 activation [165]. In a murine model, microbial translocation and release of related patterns as a result of gut barrier leakage enhanced platelet pro-aggregating capacity and thrombus growth by inducing VWF synthesis; this effect was mediated *via* TLR2 activation in the hepatic endothelium[166]. Restoration of endothelial function through L-arginine supplementation was followed by improved outcomes in hospitalized patients with COVID-19[167]. L-arginine, a semi-essential amino acid, stimulates endothelium relaxation by serving as a substrate for the synthesis of nitric oxide by NOS. It also plays a role in immunomodulation by controlling T cell proliferation[168]. The importance of gut microbiome homeostasis in preventing immunothrombosis and inflammatory damage was evidenced in a model of acute mesenteric ischemia/reperfusion injury. More specifically, commensal bacteria mitigated LPS-induced NETosis by providing tonic stimulation of the neutrophil-intrinsic TLR4 downstream signaling [169]. Furthermore, perturbation of intestinal microflora due to SARS-CoV-2 infection and the ensuing depletion of SCFAs might enhance thromboinflammation by eliminating the inhibitory effects of butyrate on the LPS/TLR4/NF-κB pro-inflammatory pathway[170]. Alternatively, dysregulation of microorganisms involved in the metabolism of trimethylamine and its metabolite TMA N-oxide (TMAO) could contribute to the hypercoagulative state in CAC[171]. TMA is generated by gut bacterial metabolism of choline, carnitine, and betaine and is rapidly converted into TMAO in the liver. TMAO is a recognized risk factor for clotting events by enhancing platelet hyperresponsiveness, endothelial dysfunction, and NLRP3 inflammasome activation[171,172]. Indeed, a recent study revealed significant alterations in metabolites pertaining to the choline/TMAO and carnitine/TMAO pathways in COVID-19 patients; these aberrations were associated with disease symptoms and severity[173].

GUT BARRIER DYSFUNCTION: AN UNDERAPPRECIATED DRIVER OF SYSTEMIC INFLAMMATION

The critical form of COVID-19 is governed by multi-layered immune system dysregulation and hyper-inflammatory responses. Overexpression of pro-inflammatory cytokines (IL-1, IL-6, TNF-α) and chemokines (MCP-1, MIP-1β, CCL5), as well as excessive oxidative stress and hyperactivation of the complement (C3a) and coagulation (d-dimer) systems are hallmarks of severe COVID-19[55,174]. In parallel, SARS-CoV-2 infection is accompanied by a tremendous influx of microbial components and metabolites into the systemic circulation due to intestinal barrier dysfunction. Several studies have shown that critically ill patients with COVID-19 present with endotoxemia and elevated plasma levels of zonulin and LPS-binding protein (LBP), which are surrogate markers of leaky gut[55,59,108,174-177]. Additional indices of disrupted intestinal permeability, such as fatty acid binding protein 2, an intracellular protein specifically expressed in IECs, and peptidoglycan, a core element of the cell wall in Gram-positive bacteria, were also increased in COVID-19 cases[178]. Furthermore, patients with severe COVID-19 as well as those with SARS-CoV-2 post-acute sequelae (PASC) had higher circulating levels of β-glucan, a fungal cell wall polysaccharide, indicating persistent fungal translocation[55,175,179]. Interestingly, the presence of circulating bacteriome in critically ill COVID-19 patients without evidence of secondary infections further corroborates the assumption of gut-derived bacterial translocation in severe SARS-CoV-2-infection[175]. Essentially, exuberant translocation precipitates microbial-mediated myeloid activation as demonstrated by increased serum levels of sCD14 and myeloperoxidase, which correspond to monocyte and neutrophil inflammation, respectively[55,174]. Multiple studies have revealed strong and unequivocal correlations between intestinal barrier dysfunction and biomarkers of inflammation and/or immunological activation, implicating a detrimental role of gut barrier defects in triggering or enhancing hyper-inflammatory responses in severe SARS-CoV-2 infection[55,59,108,163,

174,177]. More specifically, Giron *et al*[55] reported that LBP, β-glucan, and zonulin levels were all significantly associated with higher systemic levels of IL-6. During PASC, β-glucan could directly stimulate leukocytes and promote inflammation by binding to the Dectin-1 receptor and activating NF-κB pathway[179]. In hospitalized COVID-19 patients with cardiac involvement, NLRP3 inflammasome activation and subsequent IL-18 and IL-1Ra secretion were linked with circulating LBP levels[108]. In line with this, Sun *et al*[59] demonstrated that plasma levels of LBP were associated with inflammation biomarkers (CRP, IL-6, IL-8) as well as with changes in relative frequencies of lymphocytes and neutrophils. Endotoxemia was significantly associated with TNF-α, CCL5, and MIP-1β in another cohort of COVID-19 inpatients, whereas sCD14 was negatively associated with TGF-β[174].

Furthermore, gut microbiome alterations, which become more pronounced as disease progresses, showed solid correlations with markers of inflammation and tissue injury. In particular, gut dysbiosis coincided with derangements in the serum levels of IL-10, TNF-α, CRP, erythrocyte sedimentation rate, aspartate aminotransferase, and lactate dehydrogenase in patients with COVID-19[60]. A negative correlation was found between CRP and the symbiotic microorganisms *Faecalibacterium prausnitzii* and *Clostridium butyricum*, which perish in critically ill patients[60]. The abundance of commensal bacteria (*Lachnospiraceae*, *Eubacterium ventriosum*, *Faecalibacterium prausnitzii*) was followed by an increased number of CD4+ T cells, CD8+ T cells, and NK cells[69]. Conversely, opportunistic pathogens, which are commonly overrepresented in the COVID-19-related gut microbiome, such as *Bacteroides dorei* and *Akkermansia muciniphila*, were positively correlated with pro-inflammatory cytokines, including IL-1β, IL-6, and IL-8[69]. Accordingly, the predominance of the emerging pathogen, *Burkholderia contaminans*, was accompanied by T cell anergy and complement activation[59]. In parallel, the prevalence of pathogenic species in SARS-CoV-2-induced gut dysbiosis predisposes to bacteremia, leading to secondary microbial bloodstream infections, and thus, to an increased risk of serious complications[178, 180]. It should be noted that dysbiosis induces multifaceted derangements in microbial-mediated metabolic functions, including regulation of amino acid, lipid, and carbohydrate metabolism, that could further contribute to worsening outcomes. In fact, severe COVID-19 disease is characterized by a drastic shift in the composition of gut-modulated biologically active molecules that engage in inflammation signaling and immune system activation[55,59]. The long-lasting alteration of the microbial signature due to SARS-CoV-2, characterized by the predominance of pathogenic species and activation of pro-inflammatory pathways, perpetuates intestinal inflammation and could lead to long-term complications, such as tumorigenesis and colorectal cancer[60,181]. Systemic immune activation in COVID-19, which is at least partially fueled by leaky gut, has been linked to all of the severe manifestations of the disease, including cytokine storm syndrome, ARDS, renal failure, cardiovascular events, thromboembolic disease, and neurological manifestations[182]. The co-existence of intestinal barrier dysfunction in individuals with underlying diseases, such as obesity, diabetes, colorectal cancer, or immunosuppression, could exacerbate endotoxemia and the consequent immune system overactivation, predisposing to a more severe disease course[182,183].

CONCLUSION

Although the respiratory tract is the main target organ of SARS-CoV-2 infection, severe COVID-19 is considered a complex disorder affecting multiple systems. The development of GI-related symptoms, long-lasting fecal shedding of viral RNA, and identification of the virus in human intestinal tissues have brought to the spotlight the potential effects of the GI system in COVID-19 pathophysiology. The integrity of the intestinal barrier is a *sine qua non* for the accomplishment of the diverse digestive and immunomodulatory functions of the GI tract. In this setting, SARS-CoV-2 is capable of inducing deleterious effects on the gatekeepers of paracellular transport, *i.e.*, TJs, as well as on intestinal epithelial and endothelial cells through direct ACE2-dependent or indirect immune-mediated mechanisms, or both. Apart from dismantling the mechanical structures of the mucosa, COVID-19 is accompanied by profound alterations of the intestinal microflora at taxonomic and functional levels that are associated with disease severity and the host's immune system activation. Furthermore, SARS-CoV-2 hijacks innate immune responses, principally through interference with IFN signaling, and, thus, leads to inappropriate trafficking and activation of virus-specific T and B cells. In turn, this multi-layered disruption of the gut barrier can exacerbate the underlying immunopathology of COVID-19 or precipitate serious complications.

The gut and the lungs, albeit anatomically distinct, participate in a bi-directional immunological crosstalk through their respective microbes and immune cells. A well-tuned intestinal barrier harboring a diverse community of commensal microorganisms is pivotal in modulating lung immune responses and the lung microbiome. Therefore, gut dysbiosis impairs the ability to prime vigorous immune responses in the respiratory tract to effectively contain viral infections such as SARS-CoV-2. Moreover, the gut vascular bed provides an enormous endothelial surface susceptible to SARS-CoV-2-mediated injury. Disease-activated and apoptotic endothelial cells are potent drivers of neutrophil/monocyte recruitment, platelet aggregation, and coagulation activation. In parallel, dysbiosis, endotoxemia, and systemic hyperimmune reactions shape a procoagulant state within the gut microvasculature that

possibly contributes to extraintestinal thrombotic complications or ARDS pathogenesis, which are common manifestations of severe COVID-19. A compromised gut barrier allows an excessive influx of intraluminal microbiota into otherwise sterile extraintestinal compartments. This systemic dissemination of microbial constituents and metabolites contributes, to a certain extent, to immune system activation and hyper-inflammatory responses that govern the severe form of COVID-19. Preexisting comorbidities plagued with impaired intestinal permeability, such as obesity, diabetes, cirrhosis, and autoimmune disorders, might act synergistically with SARS-CoV-2 to further aggravate endotoxemia and endotoxin-mediated immune activation, predisposing to a more complicated disease course. Modulation of the gut barrier function emerges as a promising intervention to prevent or alleviate severe COVID-19 and related complications.

FOOTNOTES

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Abstract

The coronavirus disease 2019 (COVID-19) disease was first detected in December 2019 in Wuhan, China. This disease is currently one of the most important global health problems. The novel coronavirus COVID-19 is a respiratory illness, that has caused a deadly pandemic that is spreading rapidly around the world. It is not only a respiratory system virus that causes severe lung disease, but also a systemic disease agent that can affect all systems. People with COVID-19 disease usually have respiratory signs, however, the liver disorder is not an uncommon presentation. In addition, many studies around the world have revealed that the liver is injured to various degrees in patients with severe acute respiratory syndrome coronavirus 2 disease. This review mainly focuses on the impact of COVID-19 on Liver Injury at various ages.

Key Words: Liver injury; Coronavirus disease 2019; Severe acute respiratory syndrome coronavirus 2; Minireview

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Core Tip: Studies have shown that neonates have rare evidence of liver damage, and in terms of age, they show the least amount of liver damage in the face of coronavirus disease 2019 (COVID-19) among affected people. Also, many studies reported different patterns of liver damage among children with COVID-19 much less than in adults, which is probably related to differences in their innate immune system and adaptation. The highest rate of liver damage is in adult patients and aspartate aminotransferase levels had the highest relevance with mortality compared to other indices reflecting liver injury.

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INTRODUCTION

Coronaviruses are a big family of viruses belonging to the realm Riboviria, order Nidovirales, family Coronaviridae and subfamily Coronavirinae. This virus contains an RNA genome and belongs to the Coronaviridae family[1,2]. This virus is spread in a wide spectrum of humans, other mammals, and avian species, also inducing acute respiratory infections[3]. Types of coronaviruses including HCoV-NL63, HCoV-HKU1, HCoV-229E, and HCoV-OC43 have been presented as mild virulent human viruses worldwide[4]. These viruses cause mild to severe acute respiratory illnesses in humans[3]. Coronavirus disease 2019 (COVID-19) was identified for the first time in December 2019, in Wuhan, located in the capital of Hubei Province in the People's Republic of China[1]. Coronavirus disease 2019 is an infectious illness that has caused a lethal pandemic that rapidly extends worldwide[5,6]. The signs of COVID-19 appear approximately 5.2 d after the disease and last for a minimum of 41 d and a maximum of 14 d until the end of life[4,7].

In the early stages of COVID-19, it has been found that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is not only a respiratory system virus that generates severe lung disease but a systemic disease factor that can involve all systems[8,9]. Some extrapulmonary involvement of SARS-CoV-2 disease is in organs like the liver, heart, or kidneys[10]. Many studies throughout the world have demonstrated that the liver is injured to differing degrees in patients affected by SARS-CoV-2 disease[8,9].

The liver is a vital member that is mostly responsible for the storage of glycogen and regulation of blood glucose levels, protein synthesis, metabolism of toxic substances, and very other physiological processes[8,9]. Liver dysfunction has been reported in 54% of hospitalized patients affected by COVID-19 disease, most of which are more severe in COVID-19[11]. Liver injuries have been documented in patients affected by COVID-19, and commonly have mild increasing liver enzymes range from 14% to 53%[12]. Patients with severe disease, especially those hospitalized in ICU, have shown a higher increase in transaminase enzymes than patients with mild to moderate severity[13]. Furthermore, few studies investigated the dynamic change of liver function during the COVID-19 pandemic. Also, no study to date has documented the incidence of a simultaneous increase in liver transaminases and total bilirubin levels in COVID-19 patients[14].

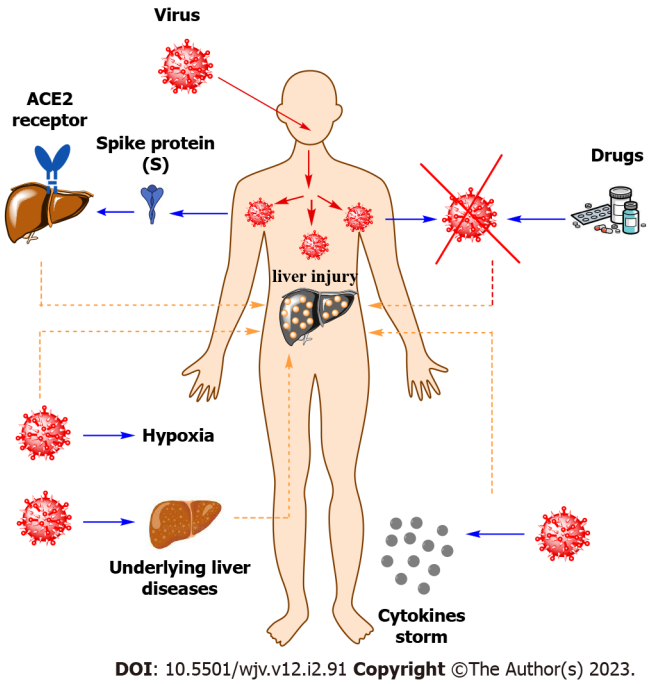
The purpose of this review is to evaluate the effect of COVID-19 on liver injury in various ages.

DEFINITION OF LIVER INJURY

Patients who make severe acute liver injury in the absence of preexisting chronic liver disease, usually indicate noteworthy liver dysfunction marked with coagulopathy, which is described as an international normalized ratio ≥ 1.5 and is classically defined as acute liver failure (ALF) when any degree of hepatic encephalopathy (HE) is existing[15]. The ALF types include: (1) Hyperacute: < 7 d; (2) Acute: 7–28 d, and (3) Subacute: 28 d to 6 mo, depending on latency between the beginning of signs and development of encephalopathy and coagulopathy[16,17].

HOW DOES COVID-19 CAUSE LIVER INJURY?

Liver injury is seen in patients with COVID-19, and its harshness is altered depending on the patient's age, geographical area, and disease severity[18]. Viral direct damage[19], immune damage, systemic inflammatory response, drug-induced, ischemia-reperfusion injury, mechanical ventilation, and underlying diseases may donate to liver injury[20] (Figure 1).



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Figure 1 Summary of liver injury in coronavirus disease 2019 patients. ACE2: Angiotensin-converting enzyme 2.

There is much evidence that COVID-19 causes abnormal liver function experiment outcomes with increased levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in people with liver damage[21,22]. Studies performed in Wuhan, China, recorded mildly elevated ALT and AST levels in 14%–53% of cases, with higher rates of both enzymes in patients with intense infection, mostly in patients requiring admission to the intensive care unit[23]. In COVID-19 patients with injured biliary tract were increased serum bilirubin, alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) levels[24]. Also, in cases where the virus causes notable liver injury and intense clinical symptoms, varying levels of ALP and GGT along with high levels of ALT and total bilirubin have been reported in 58%-78% of patients[25].

The pathophysiology of liver damage may include the cytopathic result, in which spike (S) protein of coronaviruses 2019 attaches to the angiotensin-converting enzyme 2 (ACE2) receptor, leading to reduced liver function and hepatobiliary disease[26]. S protein viral entry into the liver cells (hepatocytes and cholangiocytes), a process that involves binding to the surface of the host cell through binding of the surface unit (S1) to a receptor[27,28]. The virus attains access to the host *via* the ACE2 receptor (a type I integral membrane protein containing zinc, which indicates enzymatic action through cleaving the vasoconstrictor peptide angiotensin II to angiotensin I, a strong vasodilator peptide, therefore decreasing blood pressure). ACE2 receptor was abundantly demonstrated in epithelial cells that line a three-dimensional network of bile ducts named cholangiocytes (60%), hepatocytes (3%) in the liver, alveolar cells of the lungs, and in various organs such as the pancreas, kidney, and heart[29,30].

FACTORS RELATED TO THE COVID-19 DISEASE THAT CAUSE LIVER DAMAGE

Drugs: There are several drugs that prescribed to manage the treatment of patients with COVID-19 and associated symptoms, including therapeutic agents such as antivirals, antibiotics, acetaminophen, immunomodulators, corticosteroids, steroids, and antipyretics, that are metabolized through the liver and their use may lead to hepatotoxicity[31,32]. It has been reported that liver damage caused by these drugs is reason of anomalies in liver experiments and histological variation like micro-vesicular steatosis and liver inflammation in COVID-19 patients. Drugs like oseltamivir, arbidol, hydroxy-chloroquine, as well as ritonavir, and lopinavir in the treatment of patients may induce variable degrees of hepatotoxicity[33].

Hypoxia: Hypoxia in patients with COVID-19 is known as a major factor that causes a decrease in oxygen saturation values and finally reduction in systemic blood pressure[34]. This will ultimately cause a reduction in liver arterial perfusion *via* liver ischemia and hypoxia reperfusion injury *via* liver cell hypoxia[35].

Cytokines storm: Another factor related to COVID-19 that causes liver damage is the occurrence of a cytokine storm. In cases of the moderate and severe phase of the disease, which includes endothelial damage, it is related *via* a strong immune response to the SARS-CoV-2 virus[36]. This step is accompanied by the stimulation of inflammasomes (cytosolic multiprotein oligomers) that are responsible for the activation of caspase-1 and the release of pro-inflammatory cytokines [Interleukin (IL)-1β, IL-6, and IL-18][37]. In the next step, these cytokines stimulate the expression of genes relevant to the immune response, and through intracellular signaling, especially using IL-6, other pro-inflammatory cytokine biomarkers like tumor necrosis factor-alpha, IL-2, IL-8, IL-10, IL-17, granulocyte colony-stimulating factor monocyte chemoattractant protein, and interferon-inducible protein[38]. In addition, IL-6 activates numerous downstream signal pathways using creating complexes with its receptor[39], and also the reason for raised ferritin and C-reactive protein levels, reduced lymphocytes and enhanced neutrophils[40].

Underlying liver diseases: Underlying liver diseases can aggravate liver damage in the face of COVID-19. The prevalence of underlying liver diseases in patients with COVID-19 has been reported to be between 3% -11% in large observational studies[27,41,42]. From cases of these underlying diseases can be mentioned chronic liver disease and cirrhosis, non-alcoholic fatty liver disease, and liver transplantation[27].

THE ASSOCIATION DIFFERENT AGES AND LIVER INJURY CAUSED BY COVID-19

Many studies have demonstrated various patterns of disease and their outcomes between adults and children, possibly associated with the difference in their innate and adaptive immune systems. Children with or without chronic sickness are less likely to have a severe illness from COVID-19 confirmed in various studies[43]. However, children affected by COVID-19 have a milder infection than adults, possibly related to children having preserved effector and immunosuppressive components[44]. The differences in age, gender, and population are probably due to differences in immune responses and different variants of SARS-CoV-2[45]. Furthermore, children are less likely to have multiple chronic conditions than older people[44]. Children with a weakened immune system, such as liver illnesses considered at higher risk of coronavirus[43]. Some reports showed children to have higher ACE2 expression than older adults, that it conversion ang I (angiotensin I enzyme) into angiotensin 1-7 (ang 1-7) enzyme, thus ang 1-7 enzyme protecting against pulmonary capillary leak and inflammation. This issue can be the reason why children are more resistant to COVID-19 than adults. The mechanism of liver injury in cases by COVID- 19 is indistinct[46]. The liver damage associated with COVID-19 is described as any liver injury happening during the progression and treatment of this disease in cases with or without underlying liver illness[47]. The most common presentation of liver damage in patients is with COVID-19 shown by increasing liver enzymes and also decreasing Serum albumin in severe cases. However, reports of death in affected by COVID-19 patients due to severe liver injury rarely happen[48,49].

THE EFFECTS OF COVID-19 ON LIVER INJURY IN NEONATES

A clinical study of 10 neonates (including twins) to 9 born to mothers with COVID-19 showed that only two infants have thrombocytopenia accompanied using abnormal liver function[50]. Clinical Analysis of 48 Neonates Born to Mothers with COVID-19 (confirmed or clinically diagnosed) or without it accomplished by Liu *et al*[51] polymerase chain reaction (PCR) test of all neonates was negative. Evidence of vertical transmission and liver injury was not observed. Similarly, a clinical investigation of 19 neonates born to mothers with COVID-19 was investigated at Tongji Hospital, China. The COVID-19 real-time reverse-transcription–PCR Test of all neonates was negative. In this study also, vertical transfer of SARS-CoV-2 was not found[52]. Wang *et al*[53] investigated a case report of neonates with positive test results for coronavirus 36 h after birth. Nevertheless, whether this Newborn is vertical transfer from the mother to the neonate is yet to be verified. In this case, was observed a significant increase in AST and abnormalities in liver function tests. Stolfi *et al*[54] reported a neonate of vertical transmission of COVID-19 with liver injury, confirmed using an increase in serum transaminases in Italy. The positive PCR test of COVID-19 in a neonate less than 24 h after C-section probably indicates vertical transmission, therefore proposing a transplacental transfer of SARS-CoV-2. Liver damage in this neonate was created probably using a direct virus-mediated mechanism that correlated to ACE2 receptor expression, But the details are unknown. Out of 33 neonates born to mothers affected by COVID-19 in China, three cases have positive PCR tests for COVID-19. One neonate had observed increasing transaminases[55].

THE EFFECTS OF COVID-19 ON LIVER INJURY IN ADULTS

Guan *et al*[56] extracted information about 1099 patients with positive PCR tests for COVID-19 in 30 provinces in China (from 552 hospitals). Out of 1099 patients, 112 cases (with an average age of 47 years) had a slight increase of AST with mild illness, and 56 adults had a high increase of AST with severe illness. In 2020, in a national retrospective cohort study in France, Mallet *et al*[57] examined the danger of mortality after COVID-19 disease in adult with chronic liver disease. The study contained 259,110 of all adults with COVID-19 who were released from post-acute care and acute, public and private hospitals in France in 2020. From a total of 259,110 patients who were between 54 and 83 years old (average age 70 years) and 52% were men, including 10,006 (3.9%) and 15,746 (6.0%) patients with alcohol use disorders and chronic liver disease, respectively. The results of this study demonstrated that patients with uncompensated cirrhosis, primary liver cancer, and alcohol use disorders were at high risk for COVID-19 fatality, while patients with compensated cirrhosis, mild liver disease, organ, including liver transplant, or acquired depressive syndrome were not at risk of COVID-19 mortality. Overall, mortality was in 38,203 (15%) of the patients, including chronic liver disease 2,941 (19%) and 7,475 (28%) after mechanical ventilation.

In another study, Mantovani *et al*[42] evaluated the widespread outbreak of chronic liver disease among patients affected by COVID-19 with a meta-analysis of data in observational studies and investigating the association between the liver injury and COVID-19 disease. The number of 11 observational studies included 2034 adults aged between 45 and 54 years (average age of 49 years), and 57.2% were men. The results of this study revealed that the widespread outbreak of chronic liver disease was 3% and people with severe disease of COVID-19 had associated changes in liver enzymes and coagulation profiles, which were reported to be possibly due to an innate immune response to the virus. In addition, the findings of this study displayed that the gain in AST level in hospitalized severe patients was more frequent and significant than the gain in ALT, and AST levels had the highest relevance with mortality compared to other indices reflecting liver damage, and it was reported that common factors related with the increase in liver damage indicators were the enhance in the number of neutrophils, the decrease in the number of lymphocytes, and male gender. The association between liver damage and adverse events of Coronavirus disease is indistinct. In adult studies, a higher rate of liver enzymes was reported in adults with severe diseases than in milder diseases[43]. One of the limitations of this study is that it is a retrospective study, which may have inadvertently missed some studies with basic keyword searches. In addition, the mechanism of liver damage at COVID-19 patients with different ages in used studies has not been clarified. However, this study was summarized existing evidence on the effects of COVID-19 on the liver injury at various ages. Furthermore, this study might have helped in clinical diagnosis and treatment for COVID-19related liver disease.

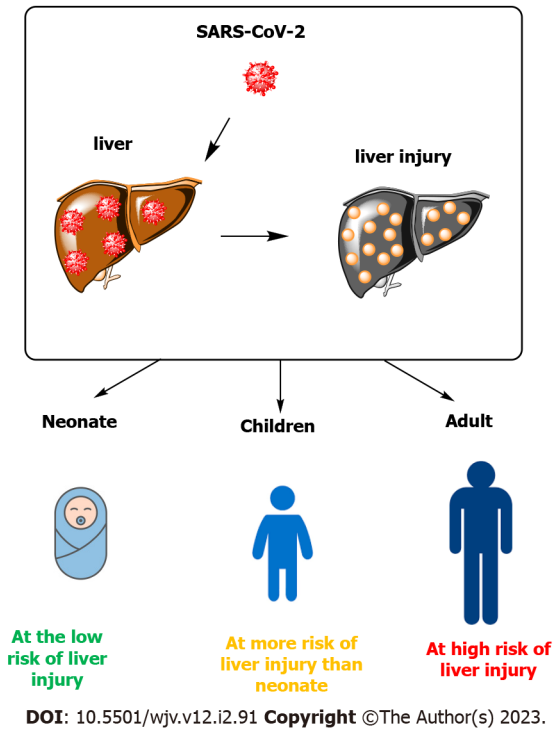
Figure 2 shows a summary of the effects of COVID-19 on Liver Injury at various ages.

RECOMMENDATIONS AND FUTURE RESEARCHES

The mechanisms of liver damage in either adults or children with COVID-19 are not fully unclear and the impact of liver injury caused by new variants of COVID-19 in patients is unexplained. Furthermore, further investigation is required to determine liver involvement and the consequence of COVID-19 on various ages with liver disease. Also, the pathogenetic mechanisms of COVID-19 on liver injury of patients in different age groups need to be investigated.

CONCLUSION

Liver damage is seen in patients affected by COVID-19, and factors including viral direct damage, immune damage, systemic inflammatory response, drug-induced, ischemia-reperfusion injury, mechanical ventilation, and underlying diseases contribute to liver injury. The association between liver damage and adverse clinical outcomes in patients affected by COVID-19 and the mechanism of SARS-CoV-2 in creating this injury is also unclear. Studies have shown that neonates have rare evidence of liver damage, and in terms of age, they show the least amount of liver damage in the face of COVID-19 disease among affected people. Most patients with COVID-19 have maintained their normal liver function during the disease, but patients with more severe disease probably had an abnormal liver function. Also, many studies reported different patterns of liver damage among children with COVID-19 much less than in adults, which is probably related to differences in their innate immune system and adaptation. Most patients with COVID-19 have a mild increase in aspartate aminotransferase, alanine aminotransferase, or total bilirubin. The highest rate of liver damage is in adult patients and AST levels had the highest relevance with mortality compared to other indices reflecting liver injury.



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Figure 2 Summary of liver injury of coronavirus disease 2019 according to the age of patients.

FOOTNOTES

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MINIREVIEWS

Immune-mediated liver injury following COVID-19 vaccination

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Abstract

Liver injury secondary to vaccination is a rare adverse event that has recently come under attention thanks to the continuous pharmacovigilance following the widespread implementation of coronavirus disease 2019 (COVID-19) vaccination protocols. All three most widely distributed severe acute respiratory syndrome coronavirus 2 vaccine formulations, *e.g.*, BNT162b2, mRNA-1273, and ChAdOx1-S, can induce liver injury that may involve immune-mediated pathways and result in autoimmune hepatitis-like presentation that may require therapeutic intervention in the form of corticosteroid administration. Various mechanisms have been proposed in an attempt to highlight immune checkpoint inhibition and thus establish causality with vaccination. The autoimmune features of such a reaction also prompt an in-depth investigation of the newly employed vaccine technologies. Novel vaccine delivery platforms, *e.g.*, mRNA-containing lipid nanoparticles and adenoviral vectors, contribute to the inflammatory background that leads to an exaggerated immune response, while patterns of molecular mimicry between the spike (S) protein and prominent liver antigens may account for the autoimmune presentation. Immune mediators triggered by vaccination or vaccine ingredients per se, including autoreactive antibodies, cytokines, and cytotoxic T-cell populations, may inflict hepatocellular damage through well-established pathways. We aim to review available data associated with immune-mediated liver injury associated with COVID-19 vaccination and elucidate potential mechanisms underlying its pathogenesis.

Key Words: Adverse effects; COVID-19 vaccines; mRNA vaccine; Autoimmune Hepatitis; Chemical and Drug Induced Liver Injury; Autoimmunity

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Core Tip: Following the worldwide implementation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination protocols, several reports suggest an increase in the occurrence of autoimmune phenomena involving the liver. Studies on vaccine-induced liver injury point to a specific pattern of hepatocellular injury that involves immune-mediated pathways. This minireview explores the underlying pathophysiology of immune-mediated liver injury following SARS-CoV-2 vaccination and examines the most widely distributed vaccine formulations' autoimmune and hepatotoxic potential.

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INTRODUCTION

As of August 4, 2022, approximately 5.3 billion people around the world have received at least one dose of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine. Widespread implementation of vaccination protocols has successfully contained the spread of the pandemic and has reduced the disease burden for patients and health systems alike[1]. Newly employed vaccination platforms, *e.g.*, mRNA- and adenovirus (AdV)- based formulations, have achieved high efficacy rates combined with a good safety profile[2]. SARS-CoV-2 vaccines have undergone the most intensive safety monitoring in the history of mankind. Both active and passive monitoring systems have been employed in order to timely detect and properly identify adverse events related to vaccination[3,4]. This worldwide vigilance has proved fruitful for epidemiological purposes and has been instrumental in ensuring public support for vaccination. Most frequently reported adverse events have been mild in nature and local in character. They primarily concern injection site-related reactions, *e.g.*, topical pain and redness or generalized systemic symptoms, like fever and fatigue[5,6]. As far as serious, organ-specific adverse events are concerned, a very low risk of myocarditis mainly in younger individuals has been linked to vaccination with an mRNA vaccine, whereas adenoviral vector vaccines have been associated with incidents of thrombosis accompanied by thrombocytopenia and possibly Guillain–Barré Syndrome (GBS) cases. A rather rare side effect that has recently come under attention is that of liver injury following vaccination with a SARS-CoV-2 vaccine.

Drug-induced liver injury (DILI), under the umbrella of which such a clinical syndrome would initially be examined, is characterized by new-onset, profound increases in liver function enzyme levels. According to the latest expert panel update, this is defined as a ≥ 5 × upper limit of normal (ULN) elevation of alanine (ALT) or aspartate aminotransferase (AST) and/or ≥2× ULN increase in alkaline phosphatase (ALP) levels or ALT/AST ≥ 3 × UNL and bilirubin ≥ 2 × ULN[7]. Upon removal of the offending agent, most cases of DILI are usually self-contained; corticosteroids are sometimes added to the therapeutic regimen if autoimmune features are demonstrated. In fact, most of the reported cases' clinical and histological features closely resemble those encountered in autoimmune hepatitis (AIH)[8,9], steering the focus of the causality investigation onto the immune-mediated background of the reaction. However, it remains unclear whether the reported association of AIH with vaccination is coincidental, represents unique SARS-CoV-2-induced antigen-specific immune activation or is associated with transient drug-induced liver injury. In this study, we aim to review underlying mechanisms driving immune-mediated liver injury following COVID-19 vaccination and discuss potential implications

METHODS

We carried out broad searches of PubMed, Scopus, and Embase between 1 January 2021 and 1 September 2022 to identify literature describing immune-mediated liver injury or autoimmune hepatitis following COVID-19 vaccination. Relevant publications were identified based on the titles and abstracts. No restriction on the type of paper or language was set, even though the main focus was put on underlying mechanisms. Two reviewers independently screened all titles/abstracts and hand-searched references of retrieved articles. Data were assessed for their quality based on overall judgement and not aggregate scores. Disagreements were discussed and resolved and duplicates were removed.

VACCINE-INDUCED AUTOIMMUNITY

Throughout the vaccine rollout period, there have been reported cases of presumed AIH that were attributed to COVID-19 vaccination because they were observed shortly after either the first or second dose of the vaccine, with the initial case described as early as January 2021[10]. AIH is part of a diverse group of chronic inflammatory liver conditions that include primary biliary cholangitis and primary sclerosing cholangitis, and its complex pathophysiology involves underlying genetic predisposition and interactions with environmental triggers[11]. Viral infections, drug exposure, and vaccinations have been implicated in the pathogenesis of AIH[12]. AIH had fallen under the radar during the phase 3 clinical trials of all vaccines, and like every other rare adverse event, much debate has ensued over its association with the vaccination. New onset autoimmune reactions following vaccination have previously been described in the literature[13]. Both Hepatitis A and Hepatitis B vaccines have been linked to the development of AIH-like conditions[14]. Human papillomavirus, Hepatitis B, and Influenza vaccines have been held accountable for autoimmune reactions[15,16]. Molecular mimicry theory has been the platform upon which causality with vaccination has been determined[17]. It has been hypothesized that individuals with a genetic predisposition to autoimmunity undergo vaccination, and similarly to other environmental inputs, *e.g.*, smoking and nutrition, their immune tolerance becomes compromised. Reportedly, susceptible groups include those with systemic lupus erythematosus, GBS, multiple sclerosis, and narcolepsy. Concerning SARS-CoV-2 vaccination, immune-based phenomena such as GBS, IgA nephropathy, immune thrombotic thrombocytopenia, and myocarditis have been linked to both novel vaccine platforms, *i.e.*, mRNA- and AdV-based formulations[18].

VACCINE-INDUCED LIVER INJURY

As of early 2021, case reports of documented AIH following COVID-19 vaccination have begun to emerge[19-21]. We estimate that AIH related to COVID-19 is almost 1 in 14 million, even though we do acknowledge that many cases remain undocumented[19]. Data mostly deriving from comprehensive case-series reporting liver injury following vaccination with SARS-CoV-2 vaccines, point to the fact that most cases are in fact, immune-related, with 57% of all patients displaying both autoantibody presence and IgG hyperglobulinemia. They mostly affected elderly females, with most of the reports originating from European countries, followed by the United States[20]. The mean time of symptom onset is close to three weeks following the first vaccination, with some individuals presenting as early as 3 d after and others coming in as late as a month later, suggesting some heterogeneity in the underlying response mechanism. The mean duration between receiving the first or second vaccine dose and subsequent onset of liver injury was 17.3 (11.2–23.4) days and was mostly associated with mRNA vaccines, possibly to their stronger immunogenic potency[20]. The presence of underlying autoimmune diseases (*e.g*, Hashimoto thyroiditis, primary sclerosing cholangitis) is evident in approximately 25% of patients and could explain temporal and spatial differences in manifestations and prevalence, respectively, according to genetic predispositions[22]. Antinuclear antibody (ANA) was by far the most prevalent autoantibody, followed by spinal muscular atrophy and anti-myocardial antibody (AMA), resembling a type 1 AIH pattern. Biopsy findings were also consistent with AIH in most individuals. Around 1/3 of those who did not undergo the diagnostic procedures fit the clinical profile of AIH. Icteric manifestations, including jaundice, choluria, and pruritus, account for around 2/3 of all presentations. Outcomes were similar in all three vaccine products, *i.e.*, BNT162b2, mRNA-1273 and ChAdOx1 nCoV-19. Although recovery time varied greatly among study populations, the mean time for transaminase normalization was calculated at 46 d[21]. Corticosteroid treatment proved safe and effective for all those who were prescribed. No relapse was noted in the subgroup of patients whose immunosuppressant treatment was discontinued and remission was maintained in all those who spontaneously recovered.

CAUSALITY OR CASUALITY

Establishing causality is by definition, a difficult task, while the mechanism of action of such a reaction remains elusive. Several theories have been proposed in an attempt to link clinical manifestations of hepatocellular injury to patterns of immune mediation involving vaccine ingredients and products. Molecular mimicry-based reactivity and pro-inflammatory interactions involving the SARS-CoV-2 spike protein have been explored (Figure 1)[23]. The vaccine adjuvants have also come under scrutiny. The BNT162b2 and mRNA-1273 vaccines employ lipid nanoparticle (LNP) coated mRNA technology, whereas the ChAdOx1 nCoV-19 vaccine is deoxyribonucleic acid based and utilizes AdV vectors. Both AdV and mRNA vaccine platforms are newly licensed; hence, many rare *in vivo* interactions are to be explored and clarified. A deeper look into the active ingredients of the vaccines may provide us with a plausible mechanism. The mRNA itself has been carefully designed and tested as to its immunogenic properties[24,25]; however, prior to translation, it may still be recognized by cytosolic and endosomal toll-like receptors. The encoded S-spike protein elicits a strong immune reaction that involves the

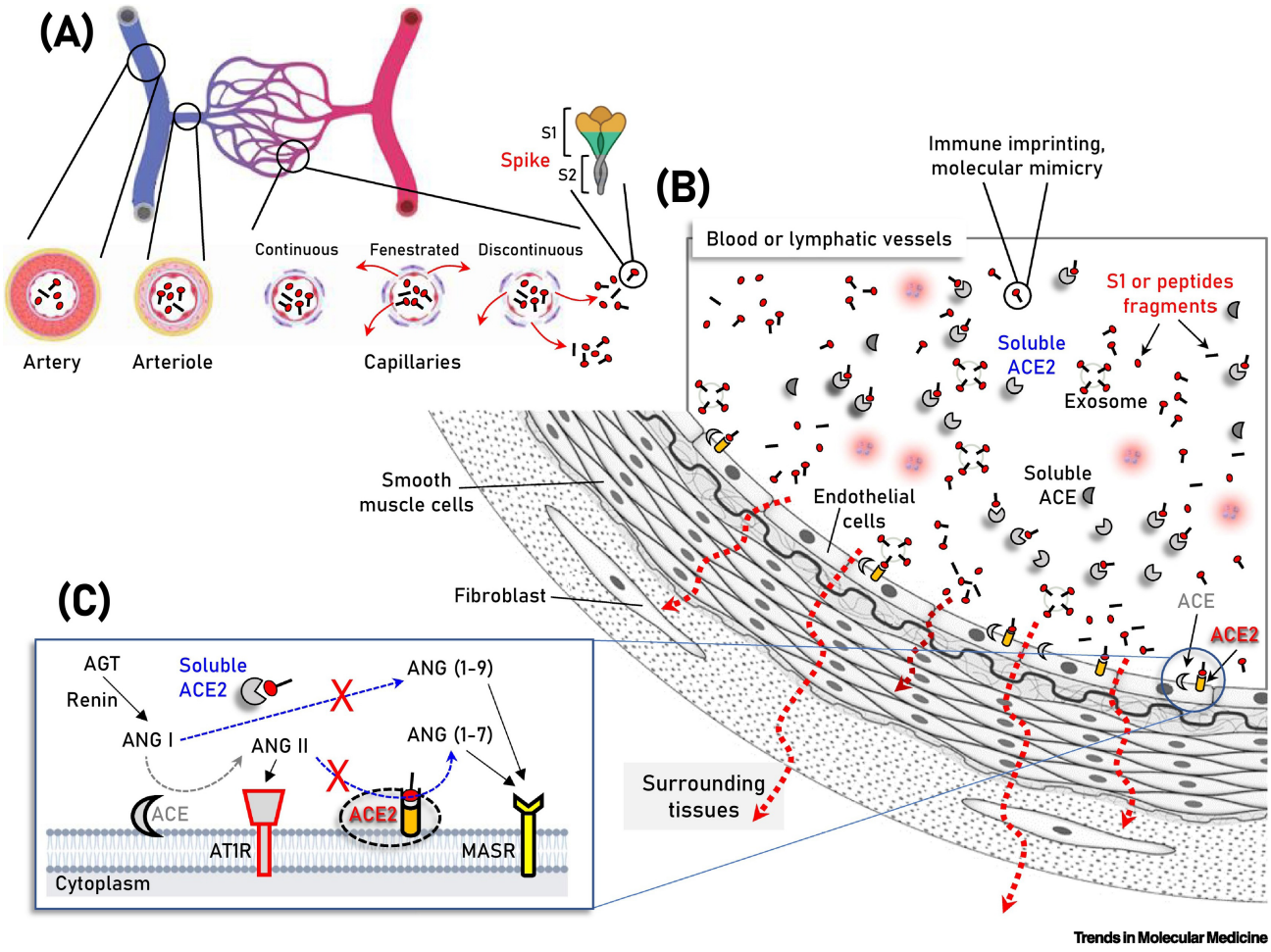


Figure 1 Schematic of the vasculature components showing vaccination-produced S protein/subunits/peptide fragments in the circulation, as well as soluble or endothelial cell membrane-attached angiotensin-converting enzyme 2. A: Parallel to immune system activation, circulating S protein/subunits/peptide fragments; B: Binding to angiotensin-converting enzyme 2 (ACE2) may occur not only to ACE2-expressing endothelial cells, but also in multiple cell types of the vasculature and surrounding tissues due to antigen diffusion (*e.g.*, in fenestrated or discontinuous capillary beds) (A, red arrows). These series of molecular events are unlikely for any severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-related antigen in the absence of severe coronavirus disease 2019, where SARS-CoV-2 is contained in the respiratory system; C: In the two counteracting pathways of the renin–angiotensin system (RAS), namely the 'conventional' arm, that involves ACE which generates angiotensin II (ANG II) from angiotensin I (ANG I), and the ACE2 arm which hydrolyzes ANG II to generate angiotensin (1–7) [ANG (1–7)] or ANG I to generate angiotensin (1–9) [ANG (1–9)] are depicted. ANG II binding and activation of the ANG II type 1 receptor (AT1R) promotes inflammation, fibrotic remodeling, and vasoconstriction, whereas the ANG (1–7) and ANG (1–9) peptides binding to MAS receptor (MASR) activate antifibrotic, anti-inflammatory pathways and vasodilation. Additional modules of the RAS (*i.e.*, renin and angiotensinogen, AGT) are also shown. AT1R: Angiotensin II type 1 receptor. Citation: Trougakos IP, Terpos E, Alexopoulos H, Politou M, Paraskevis D, Scorilas A, Kastritis E, Andreakos E, Dimopoulos MA. Adverse effects of COVID-19 mRNA vaccines: the spike hypothesis. *Trends Mol Med* 2022; 28: 542-554. Copyright ©The Author(s) 2022. Published by Elsevier.

activation of the innate inflammatory cascade, as well as that of the adaptive humoral response. Regarding the former, SARS-CoV-2 vaccines employ the type I interferon pathway in particular, in order to maintain an adequate and effective immune response, that in turn, may increase the probability of an autoimmune occurrence in certain individuals[26]. Concerning the latter, reactivity between anti-S protein antibodies and human tissue antigens has been confirmed by a recent report[27]. The systemic distribution of the spike protein has also been postulated as a mechanism to explain adverse events by mRNA-based vaccines as well. Its interaction with soluble Angiotensin-converting enzyme 2 (ACE-2) and ACE-2-ligands may point to an organ-specific pattern of insult[28]. The presentation and/or production of the spike protein by the hepatocytes may induce the activation of cytotoxic T-cell subsets. Under this scope, the formation of immune complexes cannot be excluded. Their subsequent deposition on the liver may cause inflammation or exacerbation of the underlying autoimmune disease. Matyushkina *et al*[27] have identified the susceptibility of human leukocyte antigen (HLA) B15:01 and HLA B39:01 allele carriers to autoimmunity following COVID-19. HLA B15 has been strongly associated with the development of infliximab-induced liver injury[29], while HLA B39 has been recorded as one of the most prevalent alleles in AIH patients in Pakistan[30]. In the same report, although most autoreactive antibodies were associated with nuclear products, cross-reactivity with cytokeratin 18 (CK18), a prominent liver disease biomarker, was noted. Elevated anti-CK18 antibody titers have been described in AIH patients[31], and their relationship to the soluble liver antigen (SLA) has been established in the

literature. CK18's immunoreactivity has even been proposed but disproved as a potential diagnostic marker for the SLA subgroup of AIH patients[32]. The presence of SLA antibodies has been reported twice following vaccination with the mRNA-1273 vaccine, a fact that prompted investigators to conduct a genomic sequence analysis study which revealed, however, no homology between the SARS-CoV-2 spike protein and soluble liver antigen[33].

As far as AdV vectors are concerned, their clinical application as potential gene therapy delivery particles has been hindered by their hepatotoxic properties since the start of the century[34]. Recent reports have attributed this to their inherent liver tropism[34]. It should also be noted that the recombinant ChAdOx1-S AdV used in Vaxzervria formulations is likely hepatotropic since it is derived from a subset of non-human, Y25-coded adenoviruses that have been linked to viral hepatitis outbreaks in chimpanzees in the past[34]. In the same report, the authors build a case for a post-transcriptional modification taking place inside the nucleus of AdV-transduced host cells, resulting in alternate gene splicing and subsequent truncation of produced S-protein proteins that may in turn, be released in circulation. In addition to that, they demonstrated that in ChAdOx1-S-transduced hepatocytes, the truncated S-protein is the main splicing product, thereby providing us with another plausible mechanism to explain liver injury by AdV-based formulations. Of note, the spike protein produced by Vaxzervria has comparable receptor binding selection and affinity to its original counterpart[35]. Regarding common vaccine adjuvants, CpG 1018 and Aluminum, although widely used for immune response enhancement purposes and deemed safe by clinical trials[36] and regulatory authorities alike, have the potential to induce liver injury[37] and likely precipitate the development of auto-immune disease in a small percentage of the population[38]. Reportedly, none of the vaccines discussed in this review contain the aforementioned adjuvants, but future formulations may include them. Furthermore, we need to consider other vaccine formulation specificities, like the active ingredient's delivery system. The immunogenicity of the mRNA-containing LNPs has recently come under question, despite the fact that prior to their use in COVID-19 vaccines, they were being hailed as a potential genetic treatment platform for inherited liver disease[39]. The mRNA delivery particles have been linked to the development of allergic reactions[40], suggesting a plausible, if not definite, role as immune mediators. The LNP platform mounts a strong immune response, which relies on the medium's pro-inflammatory properties for its efficacy. Such an immune response-provoking environment could potentiate a loss in self-tolerance. LNPs have been known to act as adjuvants to vaccine-induced immune reactions[41,42]. In particular, LNPs seem to trigger the NLRP3 inflammasome pathway that has been implicated in the pathogenesis of other autoimmune phenomena, like pericarditis, rheumatoid arthritis and AIH[43-46]. The intense immunogenic character of LNPs has been demonstrated both histochemically and graphically in animal models through multiple route administration of its purified form, *e.g.*, intramuscularly and intranasally.

The hepatocellular type of injury that is predominantly associated with post-vaccination liver injury can also be attributed to the direct action of cytotoxic T-lymphocytes, as rapid and sustained activation of this cell subset has been confirmed in the context of SARS-CoV-2 vaccination[47]. In a recent report, vaccination with the BNT162b2 vaccine resulted in a CD8+ rich lymphocytic infiltrate in the liver of a patient that presented with probable AIH. The clonal expansion and peripheral activation state of this particular subset of lymphocytes correlated closely with the clinical course of hepatitis in this individual, suggesting T-cells' involvement in the development and resolution of the disease[48]. It has also been demonstrated, in animal models, that cytokine-activated, "bystander" CD8+ lymphocytes may cause hepatocellular injury even in the absence of a direct antigen[49]. Accumulation of cytotoxic infiltrates in the liver has been reported in the literature following acute infection in influenza pneumonia[50]. All the plausible mechanisms resulting in immune-mediated liver injury discussed above are presented concisely in Table 1.

DIAGNOSIS

The immune-mediated mechanism of a clinical syndrome involving hepatocellular injury would most likely be highlighted by an elevation in ANA and/or AMA titers in a similar fashion to AIH. A report from early on in the pandemic noted the presence of elevated autoimmunity markers, including ANA and AMA, in SARS-CoV-2 antibody-rich plasma, thereby suggesting their self-reactive potential[51]. However, otherwise typical AIH auto-antibodies may be present in the acute phase of liver injury by multiple causes[52]. In order to distinguish between them, a biopsy is the preferred option, with fibrosis being the prime differentiating factor[53]. Features of widespread fibrosis would be evident in an AIH-stricken liver[54], whereas evidence of acute or chronic inflammation with eosinophilic infiltration between or within the portal triads is to be expected in the case of direct liver toxicity, *i.e.*, DILI[55]. It is important to note that centrilobular necrosis is not a pathognomonic clue and should not be interpreted as such[56]. All in all, a definitive diagnosis of AIH may be challenging to make, as it relies on a constellation of clinical, serological and histological findings. Response to treatment with immunosuppressants is the only way to confirm a diagnosis[57]. AIH is a chronic condition with a high relapse rate if immunosuppression is withdrawn, whereas causes closely resembling AIH do not usually relapse[58].

Table 1 Immune-mediated phenomena concerning the liver, following severe acute respiratory syndrome coronavirus 2 vaccination

Mechanism of injury	Liver antigens	Immune mediators
Molecular mimicry	SLA	Autoreactive antibodies
	CK-18	
Cytotoxicity/Humoral response	S protein (membrane expression)	Activated CD8 + clone/protective anti-S antibodies
Humoral response	ACE-2 transmembrane receptor	Protective anti-S antibodies
Immune complex deposition	S protein (soluble)	
	Soluble ACE-2/ACE-2 ligand + S protein	
"Bystander" toxicity		Activated CD8 + clone
Loss of self-tolerance/Fibrosis		Type I IFN
NLRP3 inflammasome activation		LNPs
TLR-mediated innate immune response		mRNA

SLA: Soluble Liver Antigen; CK-18: Cytokeratin 18; S protein: Spike protein; anti-S: Anti-Spike protein; ACE-2: Angiotensin Converting Enzyme-2; IFN: Interferon; LNPs: Lipid nanoparticles; NLRP3: NLR family Pyrin domain containing 3; TLR: Toll-like receptor.

CONCLUSION

Immune-mediated liver injury remains an elusive but rare entity following COVID-19 vaccination. It is the responsibility of investigators and scientists worldwide to maintain a vigilant eye and continue reporting rare incidents related to vaccination with a high index of suspicion. However, adverse events as such, are significantly less frequent than potentially serious complications of COVID-19 disease[59] and should by no means discourage vaccination programs worldwide.

FOOTNOTES

Author contributions: Akinosoglou K and Gogos C conceived the idea; Schinas G performed the literature search, reviewed data, and wrote the manuscript; Polyzou E, Dimakopoulou V, and Tsoupra S performed the literature search and reviewed data; Akinosoglou K and Gogos C critically corrected the manuscript; Akinosoglou K revised the manuscript

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Abstract

There have been numerous concerns about the disease and how it affects the human body since the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic began in December 2019. The impact of SARS-CoV-2 on the

liver is being carefully investigated due to an increase in individuals with hepatitis and other liver illnesses, such as alcoholic liver disease. Additionally, the liver is involved in the metabolism of numerous drugs used to treat comorbidities and coronavirus disease 2019 (COVID-19). Determining how SARS-CoV-2 affects the liver and what factors place individuals with COVID-19 at a higher risk of developing liver problems are the two main objectives of this study. This evaluation of the literature included research from three major scientific databases. To provide an update on the current impact of COVID-19 on the liver, data was collected and relevant information was incorporated into the review. With more knowledge about the effect of the disease on the liver, better management and therapeutics can be developed, and education can ultimately save lives and reduce the long-term impact of the pandemic on our population.

Key Words: Coronavirus; COVID-19; SARS-CoV-2; Liver; Hepatic complications

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Core Tip: We investigated the impact of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on the liver due to an increase in individuals with hepatitis and other liver illnesses, such as alcoholic liver disease. Additionally, the liver is involved in the metabolism of numerous drugs used to treat comorbidities and coronavirus disease 2019 (COVID-19). Determining how SARS-CoV-2 affects the liver and what factors place individuals with COVID-19 at higher risk of developing hepatic issues are the two main objectives of this study.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly infectious pathogenic coronavirus that appeared in late 2019 causing a pandemic of acute respiratory disease, which is known today as coronavirus disease 2019 (COVID-19)[1]. At a fast rate, the virus spread worldwide, replicated, and mutated into multiple major variants, posing a threat to global public health. SARS-CoV-2 is part of the order *Nidovirales*, family *Coronaviridae*, subfamily *Orthocoronavirinae, Betacoronavirus* genus, and *Sarbecovirus* subgenus[1,2]. It is a single-stranded, positive-sense, enveloped ribonucleic acid (RNA) virus that is 79.6% identical to SARS-CoV-2 and 96.2% like a bat-derived coronavirus strain[2]. The host receptor for SARS-CoV-2 cell entry is identical to SARS-CoV-2, the angiotensin-converting enzyme 2 (ACE-2)[3]. SARS-CoV-2 binds to ACE-2 with a higher affinity to the receptor-binding domain (RBD) of its spike protein[3]. Therefore, SARS-CoV-2 is more infectious**.** Since the first reports, which were discovered in Wuhan, China's Hubei Province, at the end of 2019, cases have been documented on every continent[3]. Globally, more than 500 million confirmed cases of COVID-19 from exposure to SARS-CoV-2 have been reported[3]. SARS-CoV-2 tends to replicate in the upper and lower respiratory tract and is transmitted by droplets and aerosols from asymptomatic and symptomatic infected subjects[4]. Most infections occur between 2-14 d (about 2 wk) with an incubation period of 5-7 d[4]. These infections tend to be uncomplicated. A small percentage of patients are hospitalized due to severe inflammation and pneumonia. Complications tend to be respiratory and multiorgan failure[4]. Risk factors for complicated diseases are older age, diabetes, hypertension, chronic cardiovascular disease, chronic pulmonary disease, and immunodeficiency[4]. The distribution of COVID-19 cases across most countries is highest in the age group of 20-59 years old[4]. Major reductions in social interactions have been implemented in many countries with SARS-CoV-2 outbreaks, leading to rapid reductions. An estimate of the infection fatality rate that is currently reported is 0.5%-1.0%[4]. Despite a rapid worldwide spread, attack rates have been lowered in most regions, demonstrating the efficacy of control measures[4].

Based on initial COVID-19 data, both healthy individuals and those with pre-existing liver disease infected with the SARS-CoV-2 virus exhibit abnormal liver function tests (LFTs), implying that the virus may play a direct role in liver damage[5]. The incidence of liver injury in patients with COVID-19 has been estimated to range from 14.8% to 53.0%[6]. A clinical study showed that patients with stable liver cirrhosis who contracted the SARS-CoV-2 virus can experience rapid deterioration as evidenced by an

increase in the Child-Pugh score[6]. The incidence of liver injury in cases of death from COVID-19 is 58.0%[6]. Liver injury following the contraction of the SARS-CoV-2 virus is characterized by hypoalbuminemia, hyperbilirubinemia, and an increase in alanine transaminase (ALT) and aspartate transaminase (AST)[5,6]. There may also be an increase in gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP), indicating injury to liver bile duct cells[6]. The degree of liver injury has a positive correlation with the severity of the infection. Mortality is statistically correlated with elevated AST and low albumin levels of 26.3-30.9 g/L[6]. The mechanism by which SARS-CoV-2 damages hepatocytes is still unclear; however, pathogenic mechanisms may include direct damage, immune-mediated, ischemia and hypoxia, thrombosis, and drug-induced[5,6]. This article aims to investigate the effects of SARS-CoV-2 on the liver and the risk factors for liver problems in coronavirus-infected patients.

METHODOLOGY

PubMed, Google Scholar, and Med Line Plus were used to conduct an electronic literature review. For the data compiled, the search was limited to peer-reviewed articles published between January 1, 2015, and July 1, 2022. The articles were chosen based on keywords such as coronavirus, COVID-19, SARS-CoV-2, and the effects of the virus on the liver. The articles were then examined and included depending on the topic's applicability.

REPORTED SYMPTOMS OF SARS-CoV-2 LIVER INJURY

Elevated LFTs in COVID-19

Altered LFTs have been observed in almost half of the hospitalized patients with COVID-19 infection [7]. In particular, elevated levels of liver enzymes glutamic-pyruvic transaminase (ALT), glutamic-oxaloacetic transaminase (AST), glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), and bilirubin have been seen to manifest as liver injury in such patients[6,8]. Previous studies have shown that the incidence of COVID-19 liver damage with elevated ALT ranges between 9.6% and 37.6%, elevated AST between 14.8 and 36.0%, and the proportion of abnormal GGT between 13.0%-24.4%[9]. These abnormal tests are the result of increased AST and ALT, whereas AST was more common than ALT. In addition, 10.5% to 69.0% of hospitalized COVID-19 patients showed abnormal LFTs. Hypoalbuminemia has also been reported as a consequence of COVID-19-related liver injury and was observed more significantly in men with COVID-19 compared to women[10]. When comparing and analyzing those with severe and non-severe COVID-19 cases, liver function abnormalities like hypoalbuminemia, GGT, aminotransferase, and bilirubin elevations were more frequent in those with severe disease as opposed to mild/moderate forms of the infection[11]. The liver injury caused by COVID-19 was related to the degree of severity of the infection and manifested as different degrees of liver function abnormalities[12].

Pathological changes in liver biopsies

Histopathological findings from liver biopsies of COVID-19 patients showed moderate microvascular steatosis with lobular and portal vein involvement[13]. Hepatocyte degeneration, with neutrophil infiltration of the hepatic lobes and sinusoidal enlargement of the central lobule, was observed. Congestion of hepatic sinuses with micro thrombosis and sinusoidal expansion, lymphocytic infiltration of the lobes, and hepatic necrosis in the periportal and centrilobular segments was also identified in patients [14]. Furthermore, pathological findings showed hepatocellular necrosis, cellular infiltration, an increase in the number of mitotic hepatocytes, and fatty degeneration[15]. In addition, COVID-19 Liver injury showed an elevation of eosinophilic bodies along with dilated hepatocytes[6]. Sinusothelial micro thrombosis disease was evident in approximately 20.0% of cases with focal endothelial damage[16]. Acinar atrophy was depicted in autopsy specimens in the late course of the infection[17]. Increased liver stiffness is also correlated with increased levels of biomarkers of liver injury, such as ALT and GGT, suggesting underlying hepatocellular and cholangiocellular damage at the biochemical level[18]. Changes in liver elasticity, viscosity, and steatosis levels were also observed in liver tissues in COVID-19 patients, with increased fibrosis compared to the control group ($P < 0.001$)[18]. In some studies, the liver appeared pale and yellowish on sectioning, with a nutmeg appearance[19]. The infection has been studied to cause cholangiocellular injury and cholestasis and consequently bile duct proliferation, with bile plug formation. In general, analyses have revealed that as the severity of COVID-19 in a patient increase, the levels of AST, ALT, total bilirubin, GGT, and ALP increase, resulting in a greater degree of liver injury, as observed in hepatocytes[20].

SARS-CoV-2 RELATED LIVER INJURY: PATHOGENIC MECHANISMS

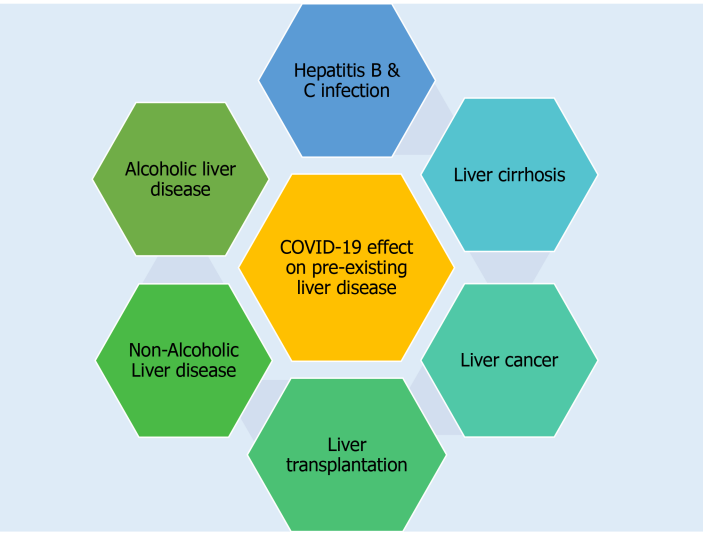
Liver injury in patients infected with SARS-CoV-2 occurs *via* several mechanisms[21]. Several studies indicate that liver injury in patients with SARS has manifested through the elevation of liver enzymes, mainly ALT and/or AST in the early stage of the infection[21]. The incidence of liver injury in SARS patients ranges from 14.8% to 53.0%[21]. One hypothesis for the cause of liver injury is a direct invasion of the hepatic parenchyma by SARS-CoV-2[21]. Autopsy of patients with SARS found a large number of virus particles in the parenchyma and vascular endothelium of the liver[21]. The main receptor used by SARS to enter cells is ACE-2 which is abundantly present in cholangiocytes, endothelial cells, and the progenitor cells of the liver[22]. This results in acute liver and hepatitis biopsies in postmortem patients showing a significant increase in macrovesicular steatosis with eosinophilic bodies and high levels of mitotic cells suggesting hepatocyte apoptosis[21]. The results suggest that SARS infection causes direct injury to the hepatic parenchyma and concomitantly compromises the regenerative capability of the liver[22]. Hepatic injury is further exacerbated by the body's immune response to severe COVID-19 infection[23]. SARS activates both the innate and acquired immune system resulting in the release of high levels of several inflammatory cytokines by immune cells[23]. The resulting cytokine storm in severe SARS infections is the cause of death in 28.0% of fatal cases of COVID[24]. Multiorgan failure is a sequela of the cytokine storm, and the liver is no exception. Critically ill patients exhibited increased levels of interferon-lambda (IFN- λ), transforming growth factor-alpha (TGF-α), thymic stromal lymphopoietin, interleukin-16 (IL-16), IL-23, IL-33, and markers linked to coagulopathy, such as thrombopoietin. Patients with severe COVID are commonly anoxic due to respiratory failure. This requires patients to be mechanically ventilated and/or on vasopressor support. Lower cardiac output has a detrimental impact on the hemodynamics of the liver[23]. The resultant reduced hepatic blood flow can lead to anoxic hypoxic hepatitis and/or cholestasis[24]. Another complication of hepatic injury due to the high levels of inflammatory cytokines released by the body includes thrombosis and vascular congestion of the liver, which have been observed in autopsy samples of patients with severe COVID-19. Patients with severe COVID-19 were found to have elevated levels of total bilirubin and ALT, as well as elevated levels of inflammatory biomarkers such as IL-6, IL-10, C-reactive protein (CRP), and D-dimer. One of the mechanisms contributing to the damage observed in the liver of these patients is due to the SARS-CoV-2 infected cells which upregulate and produce large amounts of cytokines to help combat the virus, resulting in collateral damage to both infected and uninfected cells. This hyper-stimulated systemic inflammatory response induces macro- and micro-circulatory dysfunction, leading to global hypo-perfusion resulting in hypoxia, hypo-tension, and a hypercoagulable state. Therefore, microvascular thrombosis should be considered an important cause of liver injury and dysfunction in patients with COVID-19[25]. Hepatic injury during COVID-19 infection can be exacerbated by medications leading to elevated levels of ALT and AST. Remdesivir has shown *in vitro* antiviral activity against SARS-CoV-2 and a shorter recovery time in clinical trials, but elevated hepatic enzymes have also been reported as a major adverse drug reaction. Although some studies attribute this abnormal increase to viral infection rather than the side effect of the drug, others proposed that, whether or not it was affected by SARS-CoV-2, remdesivir increased the risk of hepatotoxicity. Other commonly used drugs to treat COVID-19 such as lopinavir/ritonavir have also resulted in hepatic injury. ACE medications and angiotensin II receptor blockers, which take a more focused approach, have also been reported to raise liver enzymes in COVID-19 patients. In autopsies of COVID-19 deaths, moderate microvesicular steatosis and mild lobular and portal activity were observed and probably associated with drug-induced liver injury. Other medications used for patients with COVID-19 that can trigger liver injury include antibiotics such as macrolides and quinolones, antivirals such as ribavirin, and even steroids[26].

SARS-CoV-2 INFECTION AND PRE-EXISTING LIVER DISEASES

The effect of COVID-19 on pre-existing liver diseases discussed below are hepatitis B and C virus infection, liver cirrhosis, liver cancer, liver transplant, non-alcoholic fatty liver disease (NAFLD), and alcoholic liver disease (Figure 1).

COVID-19 effect on hepatitis B virus and hepatitis C virus infection

Hepatitis C virus (HCV) and hepatitis B virus (HBV) were reported as the leading causes of liver diseases, but no specific data on the prevalence of these infections were provided. Many studies involving patients with COVID-19 showed a relatively low prevalence of chronic liver disease (CLD) at baseline, equal to 3.0%. Similarly, a prevalence rate of 3.0% CLD is associated with documented underlying chronic HBV or HCV infections in specific populations[27]. In one study of patients admitted to hospitals for COVID-19 in the northeastern United States, HCV infection was observed in $P < 0.1\%$ of patients, but information on HCV RNA levels was insufficient. In the same study, 23 cases (3.8%) presented positive HCV serology, of whom six patients (0.99%) had detectable viral HCV load at the time of hospital admission for the diagnosis of COVID-19[27]. In contrast, 2.0% of all patients



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Figure 1 Effect of coronavirus disease 2019 and preexisting liver disease.

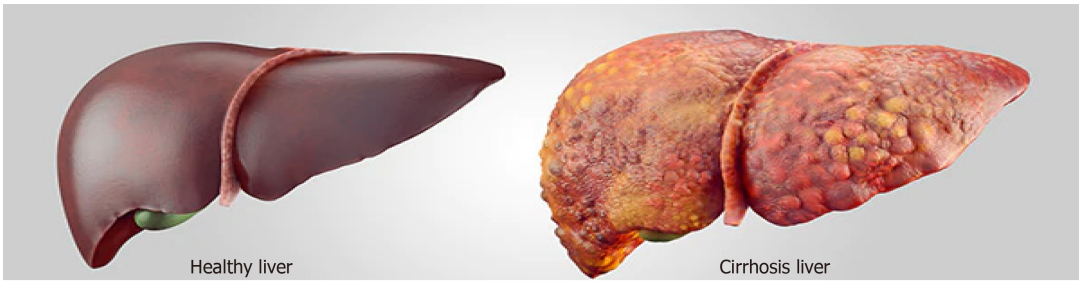
showed hepatitis B surface antigen (HBsAg) positive chronic infection[27]. The study showed patients with recorded chronic HBV or HCV infection did not experience a more severe clinical course of COVID-19 compared to patients with HBsAg negative or undetectable HCV RNA, which measured the delay in SARS-CoV-2 clearance in HBV patients. Similarly, median viral clearance was not affected by preexisting HBV or HCV infection[27]. Lastly, chronic HBV or HCV infection (in the absence of cirrhosis) did not affect the prognosis of COVID-19 in the United States population.

COVID-19 effect on liver cirrhosis

Liver cirrhosis (Figure 2) increases the mortality of SARS-CoV-2 viral infection[28]. The pathophysiological mechanism for the SARS-CoV-2 virus begins with the spike glycoprotein (S) to allow viral entry into the target cell. The virus replicates to infect other surrounding cells through the ACE-2 receptor in cholangiocytes and hepatocytes to cause biliary and liver symptoms. Elevated liver enzymes are multifactorial and strongly associated with liver injury. A prevalent hepatic phenomenon associated with SARS-CoV-2 infection presents with elevated ALT and AST levels, with abnormal ALP and bilirubin readings[29]. Elevated serum levels of GGT, a marker of hepatobiliary cell injury, are found in a quarter of patients hospitalized with COVID-19[28]. Higher levels of a hepatocellular enzyme associated with severe cases of COVID-19 directly affect mechanisms that include increased cytokine release in the viral presence or microthrombotic ischemic liver injury[29]. Although respiratory symptoms are the most reported among COVID-19 patients, these pulmonary manifestations are vulnerable to decompensated liver cirrhosis but are currently understudied. A cohort study with 250 patients with prior CLDs reported high mortality in patients with cirrhosis (RR: 4.6, 95.0%CI: 2.6-8.3)[28]. In an international study, a positive correlation was shown that patients with cirrhosis are predisposed to significant toxic liver injury due to SARS-CoV-2 infection, as acute-on-chronic liver failure had occurred in 20.0% of patients who experienced severe cirrhosis with COVID-19[28]. The viral ability of SARS-CoV-2 to bind to ACE-2 receptors on epithelial cells of the bile duct demonstrates its ability to affect liver regeneration capabilities and immune response[28]. By affecting the innate immunity of the reticuloendothelial system, the immunosuppressed state causes a cytokines-mediated reaction, resulting in liver decompression[28].

COVID-19 effect on liver cancer

Pre-existing liver diseases are considered risk factors for poorer prognosis in COVID-19 as various pathophysiological processes result in liver damage due to SARS-CoV-2 infection. Biochemical presentations of liver injury include elevated levels of ALT/AST, ALP, GGT, or total bilirubin above the normal range. The decrease in lymphocyte count and the increase in neutrophil counts demonstrate the role of innate immunity in COVID-19-associated hepatic injury. Postmortem studies in liver histology in COVID-19 patients show moderate microvascular and macrovascular steatosis with mild inflammation of the lobular portal. This highlights the pathological changes observed in hepatocellular carcinoma (HCC). Elevated levels of eosinophils are observed in autopsy studies in centrilobular steatosis, in addition to the increased number of mitotic cells[30]. Patients with HCC are closely monitored, as increased inflammation due to COVID-19 may predispose patients to post-hepatectomy liver failure. Furthermore, COVID-19 can potentially exacerbate CLD and alter treatments for cancer patients with a higher risk of infection and poor outcomes[30]. The management and monitoring of patients with HCC



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Figure 2 Healthy and cirrhosis liver.

are performed by imaging (magnetic resonance imaging, ultrasound) and measuring alpha-fetoprotein levels[29]. The practice guidelines recommend the establishment of surveillance intervals to reduce the radiologic capacity of at-risk patients, with a 98.0% estimate that at-risk patients would not develop HCC during each surveillance interval. Locoregional and systematic therapies are recommended for advanced HCC treatment. However, oral therapy with tyrosine kinase inhibitors (*i.e.*, sorafenib and lenvatinib) and immunotherapy effectively serve as first-line therapies to reduce exposure[29].

COVID-19 effect on liver transplantation

With the severity of SARS-CoV-2 infection dependent on comorbidities (*i.e.*, cardiovascular disease and diabetes mellitus), underlying liver diseases do not necessarily influence the outcome of COVID-19 infection. Solid-organ transplants, including liver transplant (LT) recipients, are increasingly susceptible to severe infections due to chronic immunosuppression, thereby increasing the risk for severe COVID-19 infection. A meta-analysis that included 17 articles and the outcomes of 1481 COVID-19 LT patients was compared with 239704 non-LT patients infected with COVID-19. From 17 articles, a cumulative incidence of mortality of 17.4% (95.0%CI, 15.4-19.6) was found among LT recipients with COVID-19 with causes of death reported as 62.54% by COVID-19-related complications (95.0%CI, 56.24-68.55), 29.88% by pulmonary failure (95.0%CI, 24.28-36), and 1.6% liver-related (95.0%CI, 0.1-2.84). Mortality was proportionate between LT and non-LT patients [OR, 0.8 (0.6-1.08); $P = 0.14$][31]. Twelve studies in the same meta-analysis reported that 23.0% of LT patients who had developed a severe COVID-19 infection were positive with symptoms that included fever (49.7%), cough (43.76%), dyspnea (29.27%), and symptoms gastrointestinal (27.26%)[31]. Eight studies from the same meta-analysis reported modification change immunosuppression in 55.9% of LT recipients infected with COVID-19[31]. Comorbidities such as hypertension, diabetes, and obesity were common in infected patients where 72.0% of the patients were hospitalized, and 16.0% required care in the intensive care unit (ICU)[31]. Although hospitalization of LT recipients far exceeded non-LT patients [OR, 1.99 (1.41-2.8), $P < 0.001$], the ICU care requirement was comparable between groups, as the cumulative incidence of graft dysfunction was 2.3% (1.3-4.1)[31].

COVID-19 effect on non-alcoholic fatty liver disease

Many observational studies have shown that patients with comorbidities such as cardiovascular disease, arterial hypertension, diabetes mellitus, CLD, or cancer are susceptible to more severe episodes of COVID-19, as seen in NAFLD and other less common disorders[32]. Recently, several meta-analyses have shown that obesity and diabetes (both strongly associated with NAFLD) are significantly associated with the progression of more severe disease and increased mortality in patients with COVID-19[32]. They have reported a six-fold increased risk of severe COVID-19 in the presence of obesity in NAFLD. In addition, a meta-analysis showed that obesity could exacerbate COVID-19 infection. Patients with severe COVID-19 disease had higher body mass indices, and obesity was associated with the development of the disease, the need for care, and admission to an ICU[32]. The risk of severe COVID-19 in obese patients was more significant than in younger patients. This suggests that NAFLD patients are at increased risk of liver damage, although liver enzyme levels at admission or during hospitalization were generally not significantly elevated. Furthermore, NAFLD was not associated with adverse clinical outcomes in younger patients with COVID-19. Another study found that NAFLD was more common in patients with severe COVID-19 than in stable patients. However, the mean age and the number of comorbidities were also significantly higher in patients with severe COVID-19 infections[32]. Patients with NAFLD had a substantially higher risk of disease progression, more likely changes in liver enzymes, and longer viral shedding times than non-NAFLD patients. Non-NAFLD patients showed that moderate to high Fibrous-4 and NAFLD fibrosis scores were strongly and independently correlated with the severe progression of COVID-19 disease[32].

COVID-19 effect on alcoholic liver disease

The ACE-2 receptor is exceedingly expressed in alveolar type II cells and liver and bile duct cells, making it significantly feasible for SARS-CoV-2 to infect cells in those areas. Especially, cholangiocytes have a specific ACE-2 receptor in more concentrations than hepatocytes, making them more susceptible to COVID-19 infection. However, because the liver harbors a widespread quantity of macrophages, generating an ample cytokine-mediated immune reaction, hepatocytes can also be prone to a SARS-CoV-2 infection. Patients with COVID-19 patients with liver cirrhosis have always shown elevated levels of ALT, AST, D-dimer, CRP, IL-6, and ferritin. Although the current literature is limited, research has proven that people with CLDs could have increased models for end-stage liver disease and undergo extended liver and pulmonary complications while infected with COVID-19. Specifically, the mortality rate in patients with preexisting liver disease is 1.8%. Lastly, the severity of liver harm due to COVID-19 infection tends to be substantially worse and more widespread in people with pre-existing alcoholic liver cirrhosis than in those without[33].

ANTI-SARS-CoV-2 TREATMENTS EFFECTS ON THE LIVER

Currently, various treatments for COVID-19 (SAR-CoV-2) are being investigated, some of which may be associated with hepatotoxicity[34]. Remdesivir (RDV), an antiviral medication, was initially developed and tested for the treatment of hepatitis C and later the Ebola and Marburg viruses. Amid the COVID-19 pandemic, RDV was approved for emergency use to treat COVID-19 in many countries[35]. In patients diagnosed with COVID-19, *in vitro* and *in vivo* studies indicate that RDV has an antiviral effect on SARS-CoV-2[36]. Various medication-related adverse events include but are not limited to reasonable degrees of nausea and vomiting, headache, fatigue, renal dysfunction, and rash[37]. The risk of an adverse event involving the liver exists as one of the clearest potential risks from RDV[38]. RDV therapy is administered intravenously for 3 to 10 d and is often accompanied by reversible mild to moderate elevations in serum AST levels, but has been rarely associated with clinically apparent liver injury. Effects on the liver range from asymptomatic to mild-with elevations in serum ALT and AST upon introduction of RDV therapy in patients with COVID-19. The systemic effects of COVID-19 likely overshadow the outcomes of hepatic involvement[37]. However, there is uncertainty regarding if the effect on liver enzymes is due to remdesivir, COVID-19 solely, or both[39,40]. It is recommended that patients remain under the supervision of health professionals to monitor liver health before and during remdesivir infusions[37]. To fully assess the risk of remdesivir-associated liver damage, more studies are necessary for this area[40]. Lopinavir (LPV) is an antiretroviral protease inhibitor, used together with ritonavir (booster) in the prevention and treatment of human immunodeficiency virus (HIV) infection[41]. Lopinavir/ritonavir (LPV/r) was developed to inhibit HIV protease, the primary distinction for the SARS-CoV-2 counterpart (3CLpro) lies within the varying spatial structure of the HIV aspartic protease as compared with 3CLpro cysteine protease[41]. The LPV/r combination improves LPV pharmacokinetics by decreasing liver metabolism by inhibiting the cytochrome (CYP) P450 3A4 enzyme[41].

A randomized controlled study in adult patients hospitalized with COVID-19 shows that of those adults treated with LPV/r, only one individual in the LPV/r group presented elevated ALT, more than 2.5 times above the normal limit[41,42]. When comparing patients treated with LPV/r to patients in the control group, there was no evidence of liver dysfunction noted in controls. It is important to acknowledge that the patient presenting with an elevated ALT had a pre-existing chronic liver condition, possibly contributing to the liver disturbance[42]. Another research study suggests that no observable side effects were found in the LPV/r group, except for transient elevation of ALT elevation (< 125 U/L) in three patients[43]. Given that none of the patients progressed to a severe clinical status at the end of the follow-up period, it is believed that LPV/r treatment rarely causes harm in patients recovering from COVID-19[42]. LPV/r is considered an independent factor for liver injury[44]. Interferons (IFNs) are natural antiviral immune modulators that help the body's immune system defend against infection and disease, including viruses and cancer[45]. Studies have shown that during SARS and middle east respiratory syndrome, Type I IFNs are markedly suppressed and the administration of exogenous Type I IFNs has been shown to reduce the severity of the symptoms of these diseases[46]. To assess the effectiveness and safety of interferon β-1a (IFN β-1a) in patients with severe COVID-19, a randomized clinical trial was conducted[47]. Comparisons were made between patients receiving IFN and those receiving controlled standard therapy. Hepatic complication rates were measured between patients in the treatment group and those patients receiving standard care while the preexisting liver disease was considered[44]. The frequency of hepatic failure did not differ between the IFN and control groups (11.90% *vs* 23.07%), suggesting that IFN-β-1a may not be a major factor in the liver damage seen by COVID-19 patients[47]. Studies show that IFNs used to treat patients with COVID-19 are unlikely to be associated with liver disease. IFNs may lead to hepatic toxicity when combined with other drugs[44]. To authenticate these results, additional studies are necessary[44]. Baricitinib is a JAK-STAT inhibitor used to treat individuals with rheumatoid arthritis who cannot tolerate more than one tumor necrosis factor (TNF) antagonist[6]. By decreasing adaptor-associated kinase 1 activity, a regulator of clathrin-

mediated endocytosis, baricitinib has been shown to affect the hyperinflammatory state that developed during SARS-CoV-2 infection and may prevent endocytosis and viral infection[6,48]. Furthermore, the oral administration of baricitinib and the excellent pharmacokinetic profile (very short half-life, low plasma protein binding, and minimal interference with CYP enzymes) make it a viable combination therapy with direct-acting antivirals such as LPV/r and RDV[48]. The growing number of reports of infections and thrombosis following the use of JAK inhibitors for the treatment of COVID-19 should be taken seriously as liver damage, cholestasis, and hepatitis unexpectedly manifested in a non-negligible fraction of individuals[6]. Furthermore, these unfavorable hepatic consequences should be evaluated. Tocilizumab is a monoclonal antibody that is used to block the inflammatory protein IL-6. Tocilizumab improves joint pain and swelling from arthritis and reduces other symptoms caused by inflammation. More recently, tocilizumab use has been indicated for the treatment of cytokine release syndrome in patients with COVID-19 infection[49]. Common side effects of tocilizumab include a runny or stuffy nose, sinus pain or sore throat, headache, or dizziness. The most common side effects of tocilizumab include headache and hypertension but, rarely, hepatotoxicity ranging from mild transaminase elevation to severe drug-induced liver injury can occur[50].

SARS-CoV-2 VACCINES EFFECT ON THE LIVER

It is not yet clear whether RNA or DNA-based vaccines have any direct effect on the liver, resulting in hepatotoxicity. Although anti-coronavirus treatments have been found to cause mitochondrial and endoplasmic reticulum dysfunction, the effects of vaccines require further testing[6]. Patients receive what are often considered benign mRNA vaccines for Crigler-Najjar syndrome and rabies, which have some form of hepatotoxicity. While there is no definitive cause, there seems to be a potential link between the two. This is not the case with DNA vaccines, which makes them strikingly different. Immune system stimulation occurs *via* a completely different mechanism than mRNA vaccines, with IFN-1 secretions triggering the immune response. Unlike mRNA vaccines, DNA vaccines do not require subsequent doses to maintain monoclonal antibody protection, making DNA vaccines not only potentially more efficacious than mRNA vaccines, but requiring lower amounts to achieve a less toxic overall therapeutic effect. More research is needed to fully understand the mechanisms involved and how they affect the liver. Existing studies exclude patients with chronic liver disease as they are contraindicated by mRNA vaccines, making little information available regarding the pathophysiology, and comparing that of otherwise healthy individuals[6]. Patients with CLD are at an increased risk of infection, which is expected given the insufficient immune response. Vaccinations are imperative to reduce mortality in patients with CLD[51]. According to the Advisory Committee on Immunization Practices (ACIP), patients with CLD should be vaccinated against SARS-CoV-2 and influenza, pneumococcus, tetanus, diphtheria, pertussis, herpes zoster, hepatitis A, and hepatitis B[52]. Specifically, with the COVID-19 vaccine, ACIP suggests an mRNA vaccine with a booster dose five months after completing the two scheduled doses. For severely immunosuppressed patients, a booster dose is recommended after three months to strengthen the immune response[53]. A double-blind randomized trial studying the administration of a third dose of the mRNA vaccine in transplant recipients showed a strong immune response compared to the placebo group[54]. In a case report of healthy patients with no history of liver disease, patients developed jaundice and elevated liver enzymes after administration of the mRNA vaccine, either after the first or second dose. In these cases, the laboratory values and symptoms resolved without treatment after several weeks. The data suggests that this response is due to a neutrophil-predominant inflammatory response[55]. Comparatively, much data shows contraindications to multiple COVID-19 vaccines, including booster doses, specific to CLD patients. Data are still needed to assess the efficacy and long-term effects of multiple COVID-19 vaccines in patients with CLD.

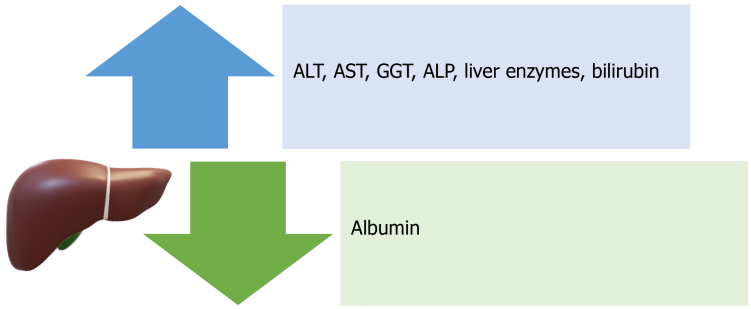
The severity of the liver disease may be assessed using the Child-Pugh scale. This scale anticipates mortality in CLD and is categorized into three stages: good hepatic function, moderately impaired hepatic function, and advanced hepatic dysfunction[56]. Multiple factors, including the stage of CLD, can be a determinant of the efficacy of a vaccine, and those in later stages are more susceptible to infections and adverse events. This is possibly due to the inefficiency of the body in producing an adequate immune response[51]. However, the World Health Organization (WHO) currently recommends that COVID-19 vaccines for those with deficient immune systems be given additional boosters to help increase a sufficient immune response[57]. It is recommended that vaccinations be administered as early in the disease process as possible to gain the best performance of the vaccine[52]. In a study on the antibody response of the vaccine in CLD patients, subjects were given the recommended series of the mRNA vaccine. The results showed that 24.0% of the subjects had a poor antibody response[58]. Additional research is needed to further assess the success of the vaccine in varying severities of CLD.

DISCUSSION

Lessons from previous coronavirus outbreaks and other viral epidemics indicate that the combination of systemic and partial inflammatory responses induced by these infections may result in severe respiratory syndromes and related complications (such as abnormal liver function, cardiac insufficiency, and renal failure)[6]. Manifestations of liver damage from SARS-CoV-2 include a decrease in albumin and an increase in ALT, AST, liver enzymes, and bilirubin[9]. Increases in GGT and ALP are also seen in COVID-19 patients, indicating liver damage to bile duct cells[7] (Figure 3). Since liver biopsies taken from a small number of COVID-19 patients did not reveal viral inclusions, but rather a macro-vesicular steatosis, liver damage may also be the result of bile duct cell damage[59]. The pathogenic alterations frequently take the form of macro-vesicular steatosis and mild lobular and portal inflammation[59,60]. According to earlier research, severe instances of coronavirus infection had a strikingly higher frequency and severity of liver impairment than moderate cases. The mechanism by which coronaviruses harm hepatocytes and influence hepatic function is still unknown, even though multiple clinical studies have shown a high link between coronaviruses and liver damage[6]. Potential mechanisms of liver injury that have been reported include immune-mediated damage because of the severe dysregulated inflammatory response, direct cytotoxicity, systemic hypoxia with hypoxic hepatitis, drug-induced liver injury, reactivation of pre-existing liver disease, mitochondrial dysfunction, SARS-CoV-2-induced hepatic steatosis, microthrombotic disease, ischemic hepatitis, cardiomyopathy with hepatic congestion, and extrahepatic release of transaminases[61]. Ischemic, hypercoagulable, and hyperinflammatory states are independent predictors of death in patients with COVID-19 and not liver injury[62]. Coronavirus infection significantly increases immunological activation. Numerous cytokines and chemokines (IL-6, IL8, IFN, and TNF, among others) are generated by immune cells after coronavirus infection and released into the blood, causing inflammation in different organs or even acute respiratory distress syndrome and multiple organ failure, suggesting that coronavirus-induced systemic inflammatory response syndrome (SIRS) and cytokine storms are important causes of liver damage[6,25]. This shows that immunotherapy is necessary for individuals with coronavirus infection, and as a result, corticosteroids and interferons are frequently utilized due to their ability to reduce inflammation[6]. Hypoxia may result in a long-term increase in reactive oxygen species, which may encourage the release of a variety of inflammatory mediators that harm the liver[6,25]. As a result, it will be important to keep an eye on patients' hypercoagulable conditions, such as thrombocytopenia and elevated levels of D-dimer and ALP, to prevent thrombosis and additional ischemia and hypoxia[6,23].

Immune compromise is typically caused by hepatitis B and C, liver cirrhosis, liver malignancy, and immunosuppressive medications after liver transplantation[6]. The severity and mortality rate in HBV infection patients are higher than in those with negative HBV due to delayed clearance of SARS-CoV-2 [6]. The Child-Pugh scores of those who have already developed liver cirrhosis are likely to rise due to liver injury caused by COVID-19[56]. Furthermore, COVID-19 complications occur earlier and to a greater extent in patients with systemic immunocompromised status[6]. COVID-19 also has a significant impact on the treatment of liver diseases[11]. The discontinuation of high-dose corticosteroid therapy in hepatitis B and C patients receiving anti-HBO treatment may result in HBV reactivation during SARS-CoV-2 infection[6]. In addition, lopinavir and ritonavir have been shown to increase the risk of developing liver injury in HBV or HCV infection patients[6,11]. Coronavirus infection is currently treated with redelivering, lopinavir/ritonavir, interferon-a, baricitinib, and tocilizumab. The difficulty in developing optimized drugs for coronavirus infection is mainly due to severe side effects. Remdesivir, lopinavir, and ritonavir have all been linked to an increased risk of liver injury, with the severity of the injury being closely related to the dose of these drugs. IFNs have the potential to trigger a non-specific immune response, resulting in hepatocyte damage and autoimmune hepatitis, as well as an increased risk of developing severe complications such as systemic inflammatory reaction syndrome and acute respiratory distress syndrome[6]. Baricitinib, as a JAK inhibitor, can increase the risk of thrombosis and cause liver damage[6,48]. Tocilizumab can also reactivate HBV in SARS-CoV-2 co-infection, causing both viral hepatitis and COVID-19 recovery to be delayed; whereas other studies have shown hepatotoxicity as a potential side effect[50]. Overall, coronavirus vaccines will be critical in preventing outbreaks, but several factors must be considered to avoid an activated innate inflammatory response, an increase in the incidence of autoimmune diseases, and vaccine-induced liver injury[6].

Furthermore, a study observed 900 patients (32.2% in the 18-39 age group, 39.7% in the 40-69 age group, and 28.1% in the 70+ age group) with SARS-CoV-2[63]. It was seen that those with comorbidities, median D-dimer, and CRP levels all increased with age. AST/ALT and ALP/GGT levels also increased significantly during COVID-19[63]. Patients with elevated hepatocellular transaminases (AST/ALT) and cholestasis parameters (ALP/GGT/bilirubin) were found in 40.3% (n = 262/650) and 45.0% (n = 287/638), respectively[61]. Importantly, patients between the ages of 40 and 69 were more likely to experience COVID-19-associated liver injury (16.0%, $P < 0.001$), abnormal liver chemistry, and liver-related death (6.5%, $P < 0.001$)[61]. After the initial SARS-CoV-2 polymerase chain reaction result was positive, elevated AST and bilirubin levels independently predicted mortality in the entire population and patients aged 40 to 69 years[63].



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Figure 3 Manifestations of liver damage from severe acute respiratory syndrome coronavirus 2. ALT: Alanine transaminase; AST: Aspartate transaminase; GGT: Gamma-glutamyl transferase; ALP: Alkaline phosphatase.

CONCLUSION

The incidence of liver injury in patients with COVID-19 has been estimated to be as high as 53.0%. Those affected by COVID-19-associated liver injury generally fall between the ages of 40 and 69. The mechanism by which SARS-CoV-2 damages hepatocytes is still unclear. However, the SARS-CoV-2 virus may play a direct role in liver damage because both healthy individuals and those with preexisting liver disease exhibit abnormal LFTs. The liver injury caused by COVID-19 is related to the degree of severity of the infection and manifests itself with different degrees of liver abnormalities. The degree of liver injury manifested by AST, ALT, total bilirubin, GGT, and ALP has been shown to have a positive correlation with the severity of the disease. Interestingly, some studies have even shown that mortality correlates with elevated AST and low albumin levels. Furthermore, SIRS and cytokine storms augment liver injury and dysfunction. Commonly used medications may play a role in liver hepatotoxicity, however further studies are necessary. Pre-existing liver diseases are considered risk factors for worse prognosis in COVID-19, specifically, liver cirrhosis was shown to increase the mortality in these patients. Although vaccines have significantly changed the course of this pandemic, CLD is a contraindication of multiple COVID-19 vaccines. However, the increased severity of liver disease in determining the immune response to the COVID-19 vaccine is still unclear, and more studies are required in this area. As more information about the virus becomes available, it will be critical to comprehend the pandemic's effects on the liver, as well as the possible long-term consequences, especially in the immunocompromised population.

FOOTNOTES

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ORIGINAL ARTICLE

Observational Study

Demographic and risk characteristics of healthcare workers infected with SARS-CoV-2 from two tertiary care hospitals in the United Arab Emirates

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Abstract

BACKGROUND

Understanding the transmission dynamics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection among healthcare workers (HCWs) and their social contacts is crucial to plan appropriate risk-reduction measures.

AIM

To analyze the socio-demographic risk factors and transmission of SARS-CoV-2 infection among HCWs in two tertiary care hospitals in Dubai, United Arab Emirates.

METHODS

The demographic and clinical characteristics were available for all HCWs in both facilities from the human resources department. A cross-sectional survey was conducted from January-April 2022 among HCWs who tested positive through

Reverse Transcriptase Polymerase Chain Reaction of the nasopharyngeal swab for SARS-CoV-2 between March 2020 and August 2021 in two tertiary-level hospitals. The survey included questions on demographics, work profile, characteristics of coronavirus disease 2019 (COVID-19), and infection among their household or co-workers. The survey also checked the knowledge and perception of participants on the infection prevention measures related to SARS-CoV-2.

RESULTS

Out of a total of 346 HCWs infected with SARS-CoV-2, 286 (82.7%) HCWs consented to participate in this study. From the sample population, 150 (52.5%) of participants were female, and a majority (230, 80.4%) were frontline HCWs, including 121 nurses (121, 42.4%). Only 48 (16.8%) participants were fully vaccinated at the time of infection. Most infected HCWs (85%) were unaware of any unprotected exposure and were symptomatic at the time of testing (225, 78.7%). Nearly half of the participants (140, 49%) had co-infection among household, and nearly one-third (29.5%) had co-infection among three or more household. Another 108 (37.8%) participants reported cross-infection among co-workers. The frontline HCWs were significantly more infected (25.1% *vs* 8.6%, $P < 0.001$) compared to non-frontline HCWs. Another significant risk factor for a high infection rate was male sex ($P < 0.001$). Among the infected frontline HCWs, a significantly higher proportion were male and shared accommodation with family ($P < 0.001$). COVID-19 vaccination significantly reduced the infection rate (83.2% *vs* 16.8, $P < 0.001$) among HCWs. Most participants (99.3%) were aware about importance of appropriate use of personal protective equipment. However, only 70% agreed with the efficacy of the COVID-19 vaccination in preventing an infection and severe disease.

CONCLUSION

The risk profiling of the HCWs infected with SARS-CoV-2 found that working at frontline and being male increase the rate of infection. COVID-19 vaccination can effectively reduce the rate of transmission of SARS-CoV-2 among HCWs.

Key Words: Coronavirus disease 2019; Risk factors; Disease transmission, infectious; Infectious disease transmission, Professional-to-patient; Health personnel; Socioeconomic factors

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Core Tip: The healthcare workers (HCWs) are vulnerable to infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In the current study, the authors found that the frontline and male HCWs were at higher risk of infection. Among the infected frontline HCWs, a significantly higher proportion were male and staying in a rented accommodation with family. The coronavirus disease 2019 vaccination is effective in preventing the transmission of SARS-CoV-2 among HCWs. This information can be utilised for the healthcare workforce management and to formulate strategies to mitigate the risk of transmission of SARS-CoV-2 to the HCWs.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has overwhelmed the healthcare resources across the globe. Since the inception of the pandemic, reports have been published on the increased vulnerabilities of healthcare workers (HCWs) compared to the general community for infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1,2]. A prospective cohort study conducted among 99795 HCWs reported that the HCWs are at a threefold higher risk of acquiring COVID-19 compared to the general community. However, the risk of exposure is not uniform and depends on multiple factors, such as the nature of work (frontline), race or ethnicity (Black, Asian, and other ethnic minorities), and access to or reuse of the personal protective equipment (PPE)[1]. Besides the risk of illness, the HCWs are at considerable risk of adverse mental health during the COVID-19 pandemic[3]. Moreover, the social and household contacts of the HCWs are also potentially vulnerable to SARS-CoV-2 infection[4].

On the other hand, the absenteeism of HCWs from work is further detrimental to the already stretched healthcare services during the pandemic[5].

From the start of COVID-19 pandemic, the experts strongly expressed concerns regarding the nosocomial transmission of SARS-CoV-2[6,7]. HCWs were assumed to play a pivotal role in the transmission chain during a nosocomial outbreak of SARS-CoV-2. However, limited information exists on the transmission characteristics and dynamics of SARS-CoV-2 infection among the HCWs or their social contacts. In this scenario, it is crucial to explore the dynamics of SARS-CoV-2 transmission among the HCWs and their social contacts to develop and implement appropriate risk-reduction measures[6].

In the current study, the authors performed a retrospective analysis of HCWs infected with SARS-CoV-2 to analyze the socio-demographic risk factors and the characteristics of SARS-CoV-2 infection among HCWs and their social contacts.

MATERIALS AND METHODS

The demographic and clinical characteristics were available for all HCWs in both facilities from the human resources department. A cross-sectional survey was conducted between January and April 2022 among the HCWs who tested positive for reverse transcriptase polymerase chain reaction (RT-PCR) for SARS-CoV-2 between March 2020 and August 2021 in two multi-specialty tertiary-level hospitals located in Dubai. The cross-sectional survey was conducted to extract further information from the infected HCWs on their social contacts, including household. The survey included Multiple-Choice Questions and questions with 5-point Likert scale. The survey questionnaire, attached in the Supplementary material, has a total of three sections: (1) Demographic details of the participants, including age, gender, department, nature of work, and COVID-19 vaccination status; (2) Details about SARS-CoV-2 infection, the reason for RT-PCR testing, severity and duration of the symptoms, and infection among their household contacts and co-workers; and (3) Knowledge and perception among the participants on PPE and infection prevention measures related to SARS-CoV-2. The human resource department, who were not part of the data analysis, sent the survey questionnaire through e-mail. The identity of the participants was kept confidential. Frontline HCWs were those who provide care for patients with COVID-19 or worked in areas with direct patient contact during the pandemic. As per the local health regulatory requirements, the HCWs were tested with RT-PCR only in case of symptomatic infections, contact tracing, or pre-travel screening during this period. The study considered only the first SARS-CoV-2 infection for further analysis. In the United Arab Emirates (UAE), seven COVID-19 vaccines were approved for use and are made available to the public for free of cost. The data on the average number of new cases in the community was extracted from the website of National Emergency Crisis and Disaster Management Authority, UAE (https://covid19.ncema.gov.ae/en). The study was approved by the scientific and ethical committee of the hospital and Dubai Scientific Research Ethics Committee (DSREC/09/2020_32).

Statistical analysis

Descriptive statistical analysis was conducted for frequencies, percentages, medians, and ranges. Continuous data was presented as mean [standard deviation (SD)] or median with Interquartile Range (IQR). Two groups were compared in this study using the 2-sample test for equality of proportions with continuity correction (Chi-square). A comparison was made between the categorical paired data with McNemar Test. The authors used Fisher exact test to compare less than five-count cells. All the tests were 2-tailed, and $P < 0.05$ was considered to be significant. The statistical analyses were conducted using R version 3.4.2 from the Comprehensive R Archive Network (R Core Team, 2020).

RESULTS

Out of a total of 1568 HCWs working in both hospitals, 346 (22.1%) tested positive for SARS-CoV-2 RT-PCR during the study period. Amongst this study population, 16 (4.6%) HCWs were found to be re-infected with SARS-CoV-2. However, as mentioned earlier, only the first infection was considered for the analysis. From the 346 infected HCWs, 286 (82.7%) HCWs agreed to participate in the cross-sectional survey. Amongst the participants, 150 (52.5%) were female, whereas a majority of the participants (230, 80.4%) were frontline HCWs, including 121 nurses (121, 42.4%). Only 48 (16.8%) participants were fully vaccinated at the time of infection.

Most of the participants (225, 78.7%) were symptomatic at the time of RT-PCR testing. Among the asymptomatic HCWs, 35 (12.2%) were tested for close contact tracing. Nearly half of the participants (140, 49%) had a co-infection with their household contacts. Moreover, half (48, 51.6%) of the infection in the households occurred in a single person, while nearly one-third (29.5%) had infection among three or more households. Further, 108 (37.8%) participants reported cross-infection among their co-workers (Table 1).

Table 1 Descriptive data on demographic and risk-profile of the healthcare workers infected with severe acute respiratory syndrome coronavirus 2

Variables		*n* (%)
Age, yr		36 (IQR-10)
Female		150 (52.5)
Staff travelling in hospital accommodation		454/1467 (29)
Work profile of healthcare workers	Doctor	34 (11.9)
	Nurses	121 (42.4)
	Technician	37 (12.9)
	Pharmacy	11 (3.8)
	Paramedical staff	10 (3.5)
	Non-clinical staff	73 (25.5)
Reason for testing with RT-PCR	Symptomatic	225 (78.7)
	Contact tracing	35 (12.2)
	Travel screening	8 (2.8)
	Other	18 (6.3)
Severity of COVID-19	Asymptomatic	36 (12.6)
	Mild	220 (76.9)
	Moderate	25 (8.7)
	Severe	5 (1.7)
Place of isolation	Institutional	50 (17.4)
	Home isolation	189 (66.1)
	Hospitalization	49 (17.1)
Symptom duration	< 1 wk	99 (34.6)
	1-2 wk	128 (44.8)
	2-3 wk	49 (17.1)
	> 3 wk	10 (3.5)
Pre-existing chronic illness	Diabetes Mellitus	33 (11.5)
	Hypertension	31 (10.8)
	Chronic respiratory disease	6 (2.1)
	Chronic kidney disease	2 (0.7)
	Other	4 (1.4)
	None	210 (73.5)
HCWs with households infected within 14 d	Total	140 (49)
	Earlier (3-14 d)	49 (35)
	Same time (within 2 d)	55 (39.3)
	Later (3-14 d)	36 (25.7)
Number of infected households	1	48 (51.6)
	2	18 (18.9)
	3	16 (16.8)
	> 3	12 (12.7)
HCWs with co-workers infected within 14 d	Total	108 (37.8)
	Earlier (3-14 d)	38 (35.2)

	Same time (within 2 d)	33 (30.5)
	Later (3-14 d)	37 (34.3)
Number of infected co-workers	1	23 (24.2)
	2	16 (16.8)
	3	3 (3.1)
	> 3	17 (17.9)

COVID-19: Coronavirus disease 2019; HCWs: Healthcare workers; RT-PCR: Reverse transcriptase polymerase chain reaction.

When compared between the infected and the uninfected HCWs, frontline HCWs (25.1% *vs* 8.6%, $P < 0.001$), who were males (54% *vs* 46%, $P < 0.001$) recorded a significantly high infection rate. The infection rate among the unvaccinated HCWs (83.2% *vs* 16.8%, $P < 0.001$) was nearly five times higher than those HCWs who were vaccinated against COVID-19. The study found that the type of accommodation (self-owned *vs* hospital sponsored) showed no significance effect on the infection rate (Table 2). A significantly high proportion of the infected frontline HCWs were males who stayed in rented accommodation with family ($P < 0.001$) (Table 3). Finally, the trend chart of a month-wise comparison of the infected HCWs and the average new cases in UAE showed three peaks.

The survey also tried to assess the knowledge and perception of the participants about safety precaution, vaccination, and the disease. Most of the participants were aware about the appropriate usage of PPE (99.3%) and did not agree to unprotected exposure to a patient with COVID-19 (85%). Around 74% of the participants agreed with the importance of social precautions like face mask, social distancing, and hand hygiene in preventing the SARS-CoV-2 infection. Only 70% agreed on the efficacy of COVID-19 vaccination in preventing infection or progression to the severe disease. The deficiency of PPE at the workplace was reported by 23.4% of the participants, whereas 29.7% participants wanted an improvement in the quality and availability of the PPE (Table 4).

DISCUSSION

This cross-sectional analysis of RT-PCR-positive HCWs from two tertiary care hospitals showed that frontline HCWs had a significantly higher infection rate. The study infers that being a male is a significant risk factors for getting infected with COVID-19. Among the infected frontline staff, a significantly higher proportion were male who shared their accommodation with family members. COVID-19 vaccination was effective in reducing the rate of infection among HCWs.

From the start of COVID-19 pandemic, various studies recorded a higher infection rate among the frontline HCWs. The risk was higher due to the reuse or inadequate availability of the PPEs and due to which the studies advocated strategies like access to high-quality PPEs and early COVID-19 vaccination to curb the spread of the virus[2,8]. Nearly one-fourth of the participants in this study reported insufficient access to the PPEs, while most were unaware of any unprotected exposure with COVID-19 patients. Limited access to adequate PPE has been linked with higher odds of infection[9-11]. Hence, ensuring access to high-quality PPEs for HCWs is an important workplace risk-reduction measure. The rate of infection was significantly higher among the male HCWs as found in other studies[2,12].

Around 13% of the study participants had asymptomatic infection. The number of asymptomatic infections could have been higher, if the hospitals had routine surveillance testing for the HCWs. However, the impact of the routine surveillance testing of asymptomatic HCWs in preventing nosocomial transmission of SARS-CoV-2 is unknown[13]. A consensus experts' panel recommended testing the HCWs to get tested for SARS-CoV-2 only when they are symptomatic or when they encountered unprotected exposure over routine testing[14].

Around 38% of the infected participants agreed to infection among their co-workers within 14 days of their own infection, and nearly one-fifth of them agreed to have three or more infected co-workers. Moreover, sharing accommodation with family or friends was significantly higher among the infected frontline HCWs. In the absence of epidemiological investigation and genomic sequencing, these infections cannot be segregated as an outbreak. However, the absenteeism of multiple HCWs from the same department can disrupt the services of already overwhelmed frontline departments during the pandemic. Despite various published reports on a nosocomial outbreak of SARS-CoV-2, ambiguity exists regarding the role of HCWs in initiating or amplifying the nosocomial outbreaks[6,15].

Most epidemiological research on SARS-CoV-2 infection among the HCWs has focused on transmission dynamics within the hospital setting. However, the research on the impact of social-cultural and demographic factors on the transmission of SARS-CoV-2 among HCWs is lacking. Recently, a large prospective study conducted in the United Kingdom found the effect of socio-demographic characteristics on the risk of infection among the vulnerable HCWs. The study found that

Table 2 Comparison of risk-factors among infected and non-infected healthcare workers, *n* (%)

	Uninfected HCWs, *n* = 1282	Infected HCWs, *n* = 346	*P* value
Accommodation			
Hospital sponsored	381 (29.7)	89 (25.9)	0.15
Self-owned	901 (70.3)	257 (74.1)	(χ^2 statistic value, 2.12)
Work profile			
Frontline	689 (53.7)	278 (80.4)	< 0.001
Non-frontline	593 (46.3)	67(19.6)	(χ^2 statistic value, 81.19)
COVID-19 vaccination			
Vaccinated	1198 (93.2)	58 (16.8)	< 0.001
Unvaccinated	88 (6.8)	287 (83.2)	(χ^2 statistic value, 895.49)
Sex			
Male	511 (39.9)	187 (54.0)	< 0.001
Female	771 (60.1)	159 (46.0)	(χ^2 statistic value, 22.38)

COVID-19: Coronavirus disease 2019; HCWs: Healthcare workers.

Table 3 Comparison of demographics among frontline *vs* non-frontline healthcare workers infected with severe acute syndrome coronavirus 2, *n* (%)

	Frontline healthcare workers, *n* = 238	Non-Frontline healthcare workers, *n* = 48	*P* value
Accommodation			
Shared with family	167 (70.2)	23 (47.9)	< 0.001
Shared with friends	60 (25.2)	24 (50)	(χ^2 statistic value, 31.07)
Non-shared	11 (4.6)	1 (2.1)	
Accommodation			
Self-rented	140 (58.8)	18 (11.4)	< 0.001
Hospital provided	49 (20.6)	25 (33.8)	(χ^2 statistic value, 42.68)
Others	49 (20.6)	5 (9.3)	
Sex			
Male	205 (86.1)	35 (72.9)	0.02
Female	33 (13.9)	13 (27.1)	(χ^2 statistic value, 5.17)

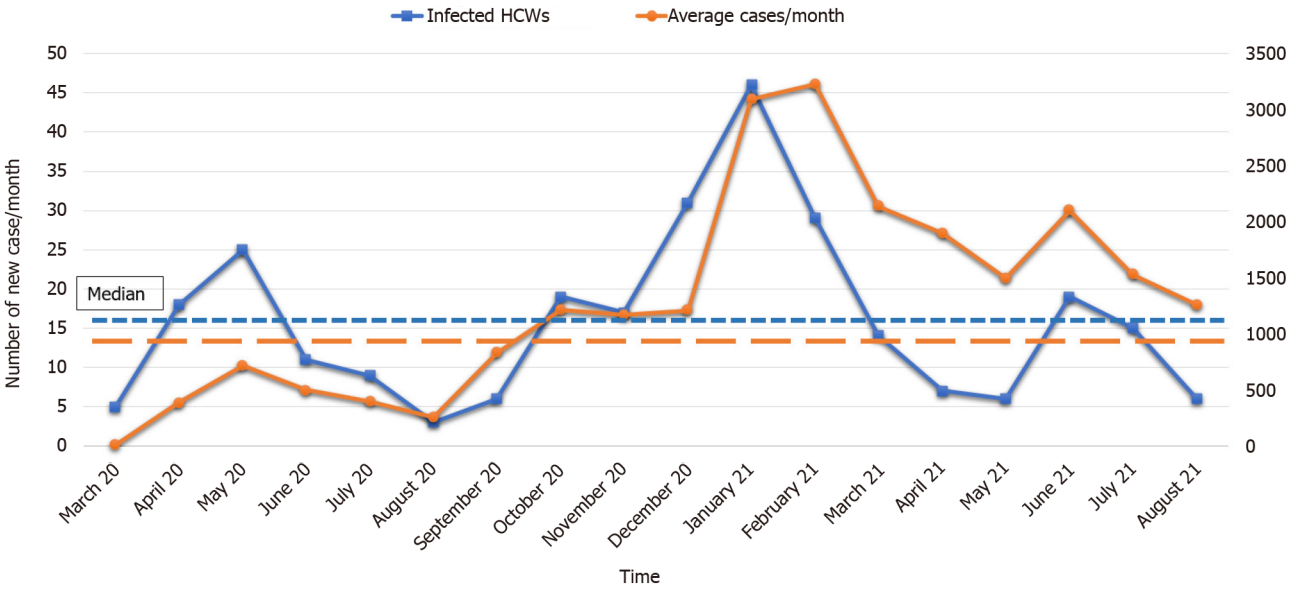
amongst the demographic and household risk factors, young age, living with a co-worker, and high religiosity are associated with high infection odds among the HCWs[9]. In another study, high odds of infection were observed among the HCWs from community contact with a suspected or a confirmed COVID-19 individual, instead of the workplace[16]. Socio-demographic risk factors may differ based on the culture and geographical differences, and the availability of resources. The cross-transmission of SARS-CoV-2 among the household is well-established concept and persists even during the low-community transmission[17].

The current study also found a significantly higher proportion of the infected frontline HCWs were staying in shared accommodation. When comparing infected HCWs per month with average new cases in the community, an agreement was observed in the peaks of two trend charts (Figure 1). This pattern reveals a synchronization in the infection rate among the HCWs and the transmission rate of SARS-CoV-2 infection in the community. Hence, the HCWs are vulnerable to contracting the infection from their households and social contacts, especially with a higher rate of SARS-CoV-2 transmission in the community. Hospital leadership can utilize this valuable insight for workforce management and to develop strategies to mitigate the risk of exposure to HCWs. Theoretically, public transport can be another risk factor for transmission. However, as reported in the literature, the current study authors did not find any increased transmission risk with public transport[18].

Table 4 Attitude and perception of the infected healthcare workers, *n* (%)

Are you aware about appropriate personal protective equipment for the care of COVID-19 patients?	Yes: 99.3%; No: 0.7%
Have you ever been exposed to a COVID-19 patient without adequate PPE?	Yes: 43 (15); No: 243 (85)
There was always enough PPE in my workplace	Agree: 183 (64); Neutral: 36 (12.6); Disagree: 67 (23.4)
PPE availability and quality should be improved at my workplace	Agree: 85 (29.7); Neutral 49 (17.1); Disagree: 152 (53.2)
Proper precautions (face mask, hand hygiene, social distance) are most important tools to save you from SARS-CoV-2	Agree: 213 (74.4); Neutral: 12 (4.2); Disagree: 61 (21.3)
Vaccines for SARS-CoV-2 can reduce infection rate and can prevent severe disease and hospitalisation	Agree: 201 (70.2); Neutral: 27 (9.6); Disagree: 58 (20.2)

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019; PPE: Personal protective equipment.



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Figure 1 Trend-chart showing the month-wise distribution of infected healthcare workers and new cases in the community. Infected healthcare workers (HCWs) are displayed in absolute numbers per month (orange color) and average cases of severe acute respiratory syndrome coronavirus 2 cases in the community (blue color). Median cases per month are displayed through dashed lines. There are three peaks observed in trend-line. The peaks coincided in the trend charts, representing an increased rate of infection among HCWs with high community transmission. HCWs: Healthcare workers.

According to a study conducted earlier, the vaccination of the HCWs effectively reduces the risk of severe disease and the transmission of SARS-CoV-2[19]. Advanced age (≥ 65 years), male sex, and other co-morbidities like diabetes mellitus, chronic respiratory disease, hypertension, chronic kidney disease, and cardiovascular disease are risk factors for severe illness and mortality[20]. COVID-19 vaccination is highly effective in reducing the progression and the severity of disease and intensive care unit (ICU) or hospital admission, especially in the elderly population and patients with co-morbidities[21]. Vaccination is an essential intervention for the HCWs to protect them from getting infected and severe illness that may require hospital or ICU admission. However, the effectiveness of the vaccine in reducing the risk of disease reduces considerably after six months of the last dose. So, a booster dose is recommended for the vulnerable population, including HCWs[19]. Vaccine hesitancy among the HCWs is a major issue in the successful implementation of the COVID-19 vaccination programme. Only 70.2% of the participants have agreed upon the efficacy of the COVID-19 vaccines. Other studies also found more vaccination hesitation among the previously infected people[22,23]. Hospital leadership and infection preventionist should address the issue of vaccine hesitancy strategically and through collaboration.

Strength and limitations

This is the first study to the best of the author's knowledge from the UAE or the countries in the Gulf Cooperation Council on risk profiling of RT-PCR-positive HCWs with COVID-19 using socio-

demographic factors. The study also evaluated the impact of COVID-19 vaccination on cross-transmission among the HCWs. The current study has a few limitations that are listed herewith. Firstly, the information on social contacts and households was collected through a cross-sectional survey. Hence, there exists a potential recall bias because of the time-gap between the period of infection and data collection. However, to avoid this bias, the data collected from the cross-sectional survey was validated through the human resource records maintained by the hospital.

There is missing data for about 17% of the eligible HCWs who did not participate in the cross-sectional survey due to reasons like resignation and immigration to other countries. Secondly, genomic sequencing was not used to confirm the phylogenetic linkage in infection among co-workers or the household. Thirdly, the small cohort size could have missed portraying the complete statistical correlation of various socio-demographic factors. Finally, the impact of the COVID-19 vaccination booster on transmission dynamics was not assessed.

CONCLUSION

The risk profiling of the HCWs, infected with SARS-CoV-2 from two tertiary care hospitals showed that the frontline HCWs had a significantly higher infection rate. Another significant risk factor was male sex. COVID-19 vaccination can effectively reduce the rate of SARS-CoV-2 transmission among HCWs.

ARTICLE HIGHLIGHTS

Research background

There is paucity of the research on the transmission dynamics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) among the healthcare workers (HCWs) and their co-workers and household. The current study conducted a retrospective analysis of the infected HCWs to analyze the socio-demographic risk factors and characteristics of SARS-CoV-2 transmission among HCWs and their social contacts.

Research motivation

HCWs are vulnerable to SARS-CoV-2 infection during their work, and the potential risk of transmission of SARS-CoV-2 infection from the household and co-workers of HCWs is unclear. This study provides valuable insights for workforce management and helps formulate strategies to mitigate the risk of exposure to the HCWs.

Research objectives

The current study evaluated the risk factors of SARS-CoV-2 infection among HCWs and explored the potential of transmission of SARS-CoV-2 among the household and co-workers of infected HCWs.

Research methods

The health records of all infected HCWs between March 2020 and August 2021 were analysed. The information on the coronavirus disease 2019 (COVID-19) vaccination, household and co-workers of the infected HCWs was collected through a cross-sectional survey.

Research results

The cross-sectional analysis of health records of 346 reverse transcriptase polymerase chain reaction (RT-PCR)-positive HCWs showed that the risk of infection was significantly higher among frontline HCWs. Being male was a significant risk factor for SARS-CoV-2 infection. Among infected frontline staff, a significantly higher proportion were male, and were staying with their families in rented accommodation. COVID-19 vaccination was effective in reducing the infection rate among HCWs.

Research conclusions

Working at the frontline and being male are the significant risk factors for SARS-CoV-2 infection among the HCWs. COVID-19 vaccination is effective in reducing the infection rate among HCWs.

Research perspectives

Future research should explore the role of community transmission of SARS-CoV-2 in the infection of HCWs.

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FOOTNOTES

Author contributions: Nasa P, Modi P, Sebutel G, Upadhyay S participated in the acquisition, analysis, and interpretation of the data; Nasa P drafted the initial manuscript; Syed T, Modi P, Sebutel G, Puspha A, Upadhyay S revised the article critically for important intellectual content.

Institutional review board statement: The study was approved by the scientific and ethical committee of the NMC Healthcare and the Dubai Scientific Research Ethics Committee (Approval No. DSREC/09/2020_32).

Informed consent statement: All involved participants of the cross-sectional survey gave their informed consent (written) prior to study inclusion.

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STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

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LETTER TO THE EDITOR

Utility of cardiac bioenzymes in predicting cardiovascular outcomes in SARS-CoV-2

Ali Osman Gulmez, Sonay Aydin

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Abstract

The relationship between coronavirus disease-19 (COVID-19) and cardiovascular diseases has been an important issue. Therefore, cardiac biomarkers and cardiac imaging have an important place in the diagnostic phase. It is important to know the relationship of biomarkers in COVID-19 so that we can understand the diagnosis of the disease, the predicted course and results after diagnosis.

Key Words: Cardiac bioenzymes; Coronavirus disease - 19; Treatment; Diagnosis; Triple rule-out computed tomography angiography; Dual energy computed tomography

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Core Tip: Recommends biomarkers, especially troponin, in patients with Coronavirus disease-19-associated myocarditis and other myocardial damage; however, they have proven that in addition to traditional biomarkers, new cardiac bioenzymes such as prepesin, copeptin also increase and significantly worsen the prognosis. Knowing this, evaluation together with other imaging methods is also important in diagnosis.

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TO THE EDITOR

We read with interest and attention the review written by Muthyala *et al*[1]. One of the main effects of the coronavirus disease 2019 (COVID-19) pandemic is on the cardiovascular system. Therefore, it is important to know and use cardiac biomarkers well, and to come to an advanced point in the diagnosis stage by combining them with cardiac imaging methods. Therefore, the authors discussed the importance of these biomarkers in COVID-19 in order to determine the ways to diagnose the disease, follow-up after diagnosis and treatment. Although biomarkers are important, we also mentioned them in our evaluation since it is important to evaluate them with imaging methods. We think that when we combine cardiac biomarkers and imaging methods, a very important point will be reached in the diagnosis.

Including troponin[2], which provides us with information about the prognosis in the diagnosis of cardiac acute coronary syndromes and myocardial damage, as well as Brain Natriuretic Peptide (BNP) [3] and pro-BNP, which gives us an advantage in the early detection of heart diseases and understanding the morbidity status of such diseases. Natriuretic peptides, especially natriuretic peptides, tend to be elevated and associated with poor prognosis in patients with heart disease, which is independently thought to be associated with COVID-19 even though the patients have no history of cardiac disease. In this review, the authors summarized the role of biomarkers in determining and diagnosing the extent of involvement of heart damage in people with COVID-19, as well as the permanent damage they may cause in the future. In the review, the researchers divided it into three main sections, considering the diagnosis, prognosis, and mortality in order to simplify the role of cardiac biomarkers in COVID-19 disease. These three sections are as follows: (1) The relationship between cardiac troponin and COVID-19-related myocardial disease; (2) The relationship between natriuretic peptides and COVID-19-related myocardial disease; and (3) The rest of the biomarkers are associated with myocardial disease.

Troponin consists of three main proteins in a complex structure[4]; Troponin C binds calcium and regulates the work of thin filaments during contraction[5]. Troponin T provides the connection of troponin in a complex with tropomyosin[6]. Troponin I acts as an inhibitory unit, and troponin C prevents contraction in the absence of calcium[7]. The amount of troponin that rises during myocardial injury has been observed to be higher among patients with COVID-19 who died compared to those who survived. In studies concentrating on this subject, a significant relationship has been shown between troponin and mortality with additional patient and hospital-related conditions, even in patients without comorbidities[8,9,10].

BNP is first proteolytically processed from its precursor proBNP to BNP. Afterwards, it is secreted from the heart as N-terminal proBNP (NT-proBNP), undivided proBNP and mature BNP and NT-proBNP in ventricular myocytes, and the amount of secretion increases in patients with heart failure [11]. In the review, it is concentrated that the main reason for the increase in natriuretic peptides in severe acute respiratory syndrome coronavirus 2 is some inflammatory processes that can lead to fulminant myocarditis. However, heart damage and hypoxia are thought to be some of the important causes of the increase in natriuretic peptides.

However, the cardiac markers mentioned in the review alone cannot rule out cardiovascular disease. Although it is supported by electrocardiogram (ECG), it may show atypical symptoms. Therefore, the differential diagnosis of acute chest pain after the new types of COVID-19 has become complicated. The viscosity increase due to COVID-19 hypoxia also causes damage to endothelial cells, resulting in increases in coagulation. In cases where biomarkers and ECG are insufficient at this stage, Triple rule-out computed tomography angiography (TRO CTA) provides us an advantage in examining the entire thoracic vascular system and detecting cardiovascular vascular diseases.

One of the important points of the new COVID-19 disease is that this disease has the potential to cause acute presentations. One of the most important of these tables is acute chest pain, which also includes respiratory tract diseases, which is the most common symptom of COVID-19. In these cases, one of the important causes of acute chest pain is diseases that affect the lung parenchyma or accompanying vascular pathologies in COVID-19 cases. In a study conducted in these cases, it is emphasized that TRO CTA is an important diagnostic method that is effective and does not require intervention to the patient in those who apply to the emergency department with sudden onset symptoms[12].

One of the important points apart from acute presentations is the long-term effects of COVID-19. In one study, persistent long-term COVID symptoms such as shortness of breath, chest pain, cough, and muscle weakness were proven to be associated with computed tomography (CT) severity values[13]. In this review, it is also emphasized that the relationship of CT with persistent symptoms yields better data than laboratory parameters. Knowing the relationship between CT severity and long-term COVID symptoms can also help to identify at-risk patients and establish follow-up programs to support these cases.

COVID-19 damages the myocardium by various mechanisms. The review focused on multiple viral infections causing sympathetic activation, direct viral invasion and proinflammatory cytokines inducing heart failure. The main reason for the increase in natriuretic peptides increased as a result of myocardial damage is thought to be due to interleukin (IL)-1β and similar proinflammatory cytokines. Magnetic

Resonance Imaging (MRI), which has an important place in the diagnosis of myocarditis, also has an important place in imaging in myocarditis formed in this way; however, Cardiac MRI has some disadvantages such as not being ubiquitous, high cost, claustrophobia, incompatibility with pacemaker and its application due to prostheses. In these cases, in a study conducted, Dual Energy CT (DECT) has proven to be significantly superior to MRI[14].

The authors recommend natriuretic peptides such as troponin and BNP in patients with myocarditis and other myocardial damage mentioned in the study; however, they proved that in addition to traditional biomarkers, new cardiac bioenzymes such as prepesin, soluble ST2 and copeptin also increase and cause marked worsening of prognosis. In addition to biochemical markers, imaging methods, especially CT, have an important place in the diagnosis of myocardial damage and comorbidities in COVID-19 patients. To give an example, DECT is used in practice as an important imaging method in the diagnosis of myocarditis-like conditions with myocardial damage[14]. TRO CTA is a frequently used imaging method in the detection of hypercoagulation, which we can give as an example of comorbidity conditions[12].

In summary, we have seen that there is a significant relationship between COVID-19 and cardiovascular system findings. After understanding this relationship, we learned that we should make the best use of the data we have at the point of diagnosis. Here, we know that we need to proceed to diagnosis by combining many cardiac biomarkers such as laboratory values such as BNP and pro-BNP with imaging methods such as ECG, CT, DECT and TRO CTA. In conclusion, we should make the best use of all available methods for diagnosis and treatment in order to reduce cardiovascular-related mortality and morbidity rates and improve prognosis in these patients.

FOOTNOTES

Author contributions: Aydin S and Gulmez AO conceived the study; Aydin S and Gulmez AO were responsible for designing, materials and supervision; Aydin S and Gulmez AO did the literature search, wrote the manuscript, and reviewed the manuscript critically; All authors have read and approved the final manuscript.

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REVIEW

Gastrointestinal tract and viral pathogens

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Abstract

Viral gastroenteritis is the most common viral illness that affects the gastrointestinal (GI) tract, causing inflammation and irritation of the lining of the stomach and intestines. Common signs and symptoms associated with this condition include abdominal pain, diarrhea, and dehydration. The infections commonly involved in viral gastroenteritis are rotavirus, norovirus, and adenovirus, which spread through the fecal-oral and contact routes and cause non-bloody diarrhea. These infections can affect both immunocompetent and immunocompromised individuals. Since the pandemic in 2019, coronavirus gastroenteritis has increased in incidence and prevalence. Morbidity and mortality rates from viral gastroenteritis have declined significantly over the years due to early recognition, treatment with oral rehydration salts, and prompt vaccination. Improved sanitation measures have also played a key role in reducing the transmission of infection. In addition to viral hepatitis causing liver disease, herpes virus, and cytomegalovirus are responsible for ulcerative GI disease. They are associated with bloody diarrhea and commonly occur in immunocompromised individuals. Hepatitis viruses, Epstein-Barr virus, herpesvirus 8, and human papillomavirus have been involved in benign and malignant diseases. This mini review aims to list different viruses affecting the GI tract. It will cover common symptoms aiding in diagnosis and various important aspects of each viral infection that can aid diagnosis and management. This will help primary care physicians and hospitalists diagnose and treat patients more easily.

Key Words: Virus diseases; Gastroenteritis; Enterocolitis; Rotavirus infections; Norovirus; Adenoviridae infections; Digestive system diseases

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Core Tip: Viral gastroenteritis is a common condition that affects the gastrointestinal (GI) tract. These viruses can affect people of all ages and are a significant public health concern. Dehydration resulting from the infection is the primary reason for emergency department visits in both children and adults. Our review discusses other GI viruses such as cytomegalovirus, herpes simplex virus, and hepatitis virus that cause manifestations such as hepatitis, gastritis, and bloody diarrhea. Both immunocompetent and immunocompromised individuals can be affected by these GI viral pathogens. Understanding the various viruses that cause GI manifestations can help with early diagnosis and appropriate management. This is a concise review of GI viral pathogens.

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INTRODUCTION

Viral pathogens cause gastrointestinal (GI) manifestations such as watery diarrhea, bloody diarrhea, and various other manifestations like hepatitis, ulcerative diseases, motility disorders, and neoplastic diseases of the GI tract in both immunocompetent and immunocompromised individuals. Infectious gastroenteritis is a major illness worldwide, especially in developing nations and viruses account for most of the illnesses. Globally, Norovirus is the leading cause of acute gastroenteritis outbreaks. Besides diarrhea, viruses are also responsible for causing diseases like hepatitis and ulcerative diseases. Asymptomatic and symptomatic co-infections with these pathogens listed are also mentioned in prior literature. Our paper is the first-of-its-kind mini-review summarizing most of the GI viral pathogens and their varied manifestations to update existing reviews[1,2]. We consolidated information on viruses that cause both diarrheal illness and non-diarrheal manifestations. Our paper will concisely summarize each entity to equip clinicians with accurate information and aid in correct diagnosis and management. As gastroenteritis is the most common disease caused by these viruses, we will be categorizing the paper based on this presentation. This categorization may also help with forming a differential diagnosis when patients present to health care centers and hospitals with the infection. We categorized the manuscript into: (1) Diarrheal illnesses further subdivided into non bloody diarrhea and bloody diarrhea; and (2) viruses associated with other non-diarrheal illnesses-hepatitis, ulcerative disease, and neoplasms (Table 1 shows common viruses that affect the GI system).

NON-BLOODY DIARRHEA FROM VIRAL GASTROENTERITIS

Norovirus

Norovirus is an RNA virus belonging to the *Caliciviridae* family and consists of forty-nine genotypes. According to Centers for Disease Control and Prevention (CDC), Norovirus is the leading cause of acute gastroenteritis among all age groups in the United States[3]. However, high-risk groups include young children, the elderly, travelers, military personnel, and the immunocompromised. On average, each year in the United States, Norovirus causes 109000 hospitalizations. Outbreaks usually occur in congregant environments like healthcare centers, cruise ships, and restaurants *via* fecal-oral transmission through food, water, or person-to-person contact. It is typically a self-limiting disease with symptoms like diarrhea, nausea/vomiting, and abdominal cramps that resolve within two to four days. It is primarily a clinical diagnosis. However diagnostic modalities include electron microscopy, polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), and immunochromatographic testing[4]. Diagnostic testing for Norovirus is rarely used given the short-lived nature of the disease. Currently, no drug has been clinically approved to treat norovirus infection owing to its mild state of disease. Complications of this illness include electrolyte imbalances, chronic gastroenteritis, irritable bowel syndrome, inflammatory bowel disease, convulsions, and encephalopathy (especially in children)[5]. Vaccinations are not currently licensed for norovirus infection prevention, but many trials are underway to assess the impact and efficacy of vaccines on disease prevention and transmission.

Rotavirus

Rotavirus, an RNA virus, and a member of the Reoviridae family is the major cause of diarrhea in children younger than five years. Reports from the Global burden of disease showed that 128530 deaths occurred due to Rotavirus infection alone[6]. Rotavirus contributed to 29.3% of total diarrheal deaths in 2015[6]. Younger age groups (< 5 years) and lower socioeconomic status seem to be the targets of the

Table 1 Common viruses affecting the gastrointestinal system

Virus	Route of infection	Population affected	Type of disease	Diagnosis	Treatment
Norovirus	Fecal-oral, fomites	Children < 5 yr	Gastroenteritis	Self-limited	Supportive
Rotavirus	Fecal-oral, fomites	Children < 5 yr	Gastroenteritis	Self-limited	Supportive
Adenovirus	Fecal-oral, fomites	Any age group, especially children	Gastroenteritis	Self-limited	Supportive
Hepatitis A	Fecal-oral, fomites	Any age groups; international travelers to endemic countries, IV drug users, men who have sex with men	Gastroenteritis, acute viral hepatitis, and fulminant liver failure	Serology	Supportive
Astrovirus	Fecal-oral, fomites	Children < 5 yr	Gastroenteritis	Self-limited	Supportive
Sapovirus	Fecal-oral, fomites	Children < 5 yr	Gastroenteritis	Self-limited	Supportive
Coronavirus	Fecal-oral, fomites	Any age group	Gastroenteritis; respiratory infection	Self-limited, viral rapid antigen, PCR can be performed	Supportive; for COVID-19-antivirals (nirmatrelvir/ritonavir, remdesivir), steroids, biologics (tocilizumab, baricitinib)
Hepatitis E	Fecal-oral, Fomites	15-40 yr	Gastroenteritis, acute hepatitis, and acute liver failure	Serology	Supportive
Cytomegalovirus	Contact with bodily fluids and organ transplantation	All ages	Colitis, toxic megacolon, and peritonitis	Viral PCR	Ganciclovir, valganciclovir, ganciclovir resistant-foscarnet, cidofovir
Herpes simplex virus	Sexual contact	MSM	Proctitis and anal ulcerations	Viral PCR	Acyclovir, valacyclovir, and famciclovir
HIV	Contact through bodily fluids like sexual contact, injection drug use, perinatal transmission	All ages	Acquired immunodeficiency syndrome, HIV enteropathy-diarrhea	2-step testing-combination antigen-antibody test followed by HIV 1/2 differentiation assay	HAART-combination therapy with Tenofovir, Emtricitabine, raltegravir, and bictegravir
Hepatitis B and D	Contact through bodily fluids like sexual contact, injection drug use, perinatal transmission	Usually adults, but all age groups can be affected	Hepatitis, cirrhosis, hepatocellular carcinoma	Serology, DNA load	Acute infections usually resolve; in some cases, tenofovir, entecavir
Hepatitis C	Contact through bodily fluids like sexual contact, injection drug use, transfusion, or perinatal transmission	Usually adults, but all age groups can be affected	Hepatitis, cirrhosis, hepatocellular carcinoma	Serology, RNA load	Combination therapy specific for genotypes; includes sofosbuvir/velpatasvir, glecaprevir/pibrentasvir
Varicella Zoster	Droplet, and contact infection	Commonly in children but can occur in any age group. Reactivation common in immunocompromised	Erosive disease of the stomach and intestines, motility problems	Clinical diagnosis, but RNA PCR is used for atypical presentations	Acyclovir, valacyclovir, and brivudin
Epstein-Barr virus	Contact with bodily fluids, especially saliva; sexual transmission, blood transfusion, organ transplantation	Individuals aged 15-24 yr	Gastritis, Enteritis, esophageal disorders, and gastric cancer	Mostly clinical diagnosis but can be diagnosed by serology	Symptomatic treatment, acyclovir in some cases
HHV-8	Sexual transmission, contact with saliva, blood transfusion, and organ transplantation	All age groups	Maculopapular and polypoid lesions of the GI tract	Endoscopy and biopsy	Radiation, intralesional chemotherapy, or systemic chemotherapy with liposomal doxorubicin and paclitaxel
HPV	Sexual transmission-oral, vaginal, anal sex	15-49 yr	Oropharyngeal, esophageal, gastric, colorectal, and anal cancers	Cytology and viral testing	Cancer-specific treatment

HIV: Human immunodeficiency virus; HPV: Human papillomavirus; HHV: Human herpesvirus 8; MSM: Men who have sex with men; PCR: Polymerase chain reaction; GI: Gastrointestinal.

disease[7]. Diarrhea caused by the virus is thought to be *via* two mechanisms: (1) Osmotic diarrhea due to malabsorption secondary to enterocyte damage; and (2) secretory diarrhea from activation of the enteric nervous system and non-structural protein-4[7]. The disease is transmitted through fecal-oral routes and fomites. Clinical manifestations include diarrhea, vomiting, and fever. Clinical morbidity is due to severe dehydration requiring hospitalization and can sometimes also lead to necrotizing enterocolitis[8]. ELISA can detect the virus until 1 wk after the onset of diarrheal illness whereas real time PCR (RT-PCR), being more sensitive can detect the virus until longer periods[7]. Fluid and electrolyte resuscitation remains the mainstay of treatment. Symptomatic management with Antiemetics and Antidiarrheal drugs decreases fluid losses thus fastening recovery and preventing death. Routine use of antivirals is not recommended for this infection. The advent of Rotarix and RotaTeq around 2006-2008 brought about a notable change in the morbidity and mortality of rotaviral disease. Mortality among children younger than 5 years of age has decreased by more than 45% since the mid-2000s[6]. It is prudent to say that, although there has been a shift from rotaviral illness being a fatal disease to a non-fatal disease, continued efforts are necessary to widen vaccination coverage and improve water and sanitation facilities.

Adenovirus

Adenovirus is a double-stranded DNA virus belonging to the Adenoviridae family. HAdV-F40 and F41 are the enteric serotypes implicated in causing acute gastroenteritis[9]. According to the re-analysis done by Global Enterics Multicenter Study, adenovirus was the second most common cause of diarrheal illness after Rotavirus in infants[9]. Infections caused by Adenovirus include febrile respiratory illness, pharyngoconjunctival fever, keratoconjunctivitis, and gastroenteritis. Transmission routes include aerosols, fecal-oral, and fomites. Adenovirus infections occur in congregate settings like daycare centers, summer camps, college campuses, and military camps. Symptoms of adenoviral gastroenteritis are like any other gastroenteritis including diarrhea, vomiting, and abdominal cramps. However, a multicenter study done in 8 Low-resource countries showed that fever was more commonly associated with adenoviral infection compared to other viral infections other than rotavirus[10]. Diagnostic methods are not often used but consist of antigen detection, PCR, and serology. No specific treatment is available for the infection but measures like rehydration, adequate food intake, and zinc supplementation are essential, especially in low to middle-income countries. Complications of adenoviruses include intussusception, hepatitis of unknown cause (adenovirus type 41), chronic lung disease, meningoencephalitis, and cystitis[11,12]. Currently, vaccination against Adenovirus type 4 and 7 is FDA-approved to prevent febrile acute respiratory disease for military populations aged 17 years to 50 years.

Astrovirus

Astroviruses are single-stranded RNA viruses belonging to the family of Astroviridae. Astroviruses are responsible for 0.5%-15.0% of diarrheal outbreaks across the world[13]. Transmission is through the fecal-oral route and fomites. Like any other gastroenteritis-causing virus, outbreaks happen in communal settings like schools, nursing homes, and swimming pools[14]. The incubation period is long (4.5 d) and it causes mild diarrhea lasting for about 2 d to 3 d associated with symptoms like fever, anorexia, and vomiting[15]. Diagnostics include electron microscopy, cell culture, immunoassays, and RT-PCR, with the latter being the most commonly used modality[16]. This infection is self-limited and usually resolves with fluid and electrolyte replacements. Immunocompromised adults and the elderly have longer-lasting symptoms with rare occurrences of complications like meningitis and encephalitis [17].

Sapovirus

Sapoviruses are single-stranded RNA viruses belonging to the same family as Norovirus, *Caliciviridae*. According to the Etiology, Risk Factors, and Interactions of Enteric Infection and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study, sapovirus had the second-highest incident rate of acute diarrhea after Shigella. The study attributed an incidence rate of 22.8 cases per 100 child years to sapovirus with a 95% confidence interval ranging from 18.9 to 27.5[18]. Transmission occurs through fecal-oral contact, contaminated food/water, and fomites[19]. They are known to cause gastroenteritis in humans, specifically aged < 5 years, and animals[20]. Outbreaks occur in communal settings like daycare centers, hospitals, nursing homes, and schools[21]. While the usual clinical symptomatology is the same as any gastroenteritis-causing virus, it has also been implicated in causing chronic diarrhea, especially in immunocompromised populations. Definitive diagnosis by PCR detection, however, is not indicated due to the self-limiting nature of the disease and the cost considerations[21]. No antivirals are warranted, World health organization (WHO) guided treatment for diarrhea viz rehydration, zinc supplementations, and adequate nutrition is considered the treatment of choice[22]. Interestingly, Nitazoxanide has been tried in transplant patients with some benefits[23]. Cases of Sapoviruses causing septic shock and intestinal obstruction have been reported in the literature[24,25].

Enterovirus

Enteroviruses are single-stranded RNA viruses belonging to the Picornaviridae family. Enterovirus

genus consists of viruses like Poliovirus, Coxsackie virus, Echovirus, rhinovirus, and other enterovirus subtypes. According to the CDC, it is estimated that around 10-15 million non-polio-enterovirus infections occur annually[26]. They can be transmitted *via* the fecal-oral route or through respiratory secretions. Infants, children, and teenagers are more likely to get affected than adults[27]. They generally have a secondary tissue tropism and spread to other target tissues after they infect the GI system[28]. Non-Polio enteroviruses are known to cause diseases like aseptic meningitis, hand-foot-mouth disease, flaccid paralysis, myocarditis, pancreatitis, *etc*[28,29]. Some non-specific GI manifestations are less common but include abdominal pain, vomiting, and diarrhea and are self-limited. Enteroviruses can be detected in stool, pharynx, blood, and CSF using techniques like PCR, serology, and cell culture. Treatment is usually symptomatic care and no targeted therapies have been developed yet. Some of the dreaded complications of enteroviruses include meningitis, encephalitis, myocarditis, and acute flaccid paralysis[30,31]. The only enteroviral vaccine is the polio vaccine to prevent poliomyelitis.

Coronavirus

Coronaviruses are single-stranded RNA viruses belonging to the Coronaviridae family. SARS-CoV-1 emerged in 2002-2003 and caused a global outbreak that affected over 8000 people in 26 countries, with a case fatality rate of approximately 10%[32]. MERS-CoV emerged in 2012 and has since caused sporadic outbreaks in the Middle East and other regions, with a case fatality rate of approximately 34%[33]. Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in late 2019 and has since spread globally, causing a pandemic that has affected millions of people worldwide. As of April 21, 2023, there have been over 435 million confirmed cases and over 5.8 million deaths reported globally[34]. Coronaviruses are transmitted through multiple routes, mainly through droplets, contact with infected persons, and contaminated surfaces.

Human Coronaviruses are known to cause respiratory infections, but they also cause GI symptoms including diarrhea, nausea, vomiting, and abdominal pain. The majority of HCoV infections like diarrhea occur in neonates, infants, and children. Also, they co-infect with other enteric viruses like norovirus and rotavirus. SARS-CoV and MERS-CoV, members of the coronavirus family have also been implicated in causing the above symptoms[35]. SARSCoV2 causing COVID-19 also presents as diarrhea, nausea, vomiting, and abdominal pain along with respiratory symptoms and sometimes even in the absence of respiratory manifestations[36]. GI manifestations were speculated to be caused due to the high expression of ACE2 receptors in the gut which is the binding site for SARS-CoV-2[37]. Changes in the gut microbiota have also been observed in COVID-19-infected patients. Although they are respiratory viruses, interestingly, the GI transmission of this group of viruses has also been proven as many studies have mentioned the detection of RNA in stool samples[35]. Currently, no specific antivirals are indicated in SARS and MERS infections, whereas COVID-19 treatment entails various therapies like antivirals, steroids, biologics, *etc*. The most common complications of COVID-19, caused by SARS-CoV-2, include pneumonia and acute respiratory distress syndrome (ARDS), which can lead to respiratory failure and death in severe cases[38]. COVID-19 can also cause a range of other complications, including cardiovascular and neurological complications, blood clots, and multisystem inflammatory syndrome in children[39]. MERS-CoV infections are also associated with severe respiratory illness and complications such as pneumonia, ARDS, and septic shock[40]. SARS-CoV-1 infections can cause similar respiratory complications, as well as complications such as liver and kidney failure[41]. Currently, several vaccines against the COVID-19 have been developed. Pfizer-BioNTech and Moderna mRNA vaccines have reported efficacies of over 90% in preventing symptomatic COVID-19[42,43].

Hepatitis E virus

Hepatitis E virus is a single-stranded RNA virus belonging to the family Hepeviridae. According to the WHO, there are an estimated 20 million HEV infections worldwide every year[44]. hepatitis E caused approximately 44000 deaths in 2015 (accounting for 3.3% of the mortality due to viral hepatitis)[44]. Hepatitis E is mainly transmitted through food and water contamination, also through perinatal transmission, and blood transfusions[45]. Pregnant women, especially those in their third trimester, and individuals with pre-existing liver disease are the most vulnerable groups for developing severe HEV infection[46-48]. Other groups that may be at increased risk of HEV infection include travelers to endemic areas, healthcare workers, and individuals who consume undercooked or raw pork[49]. It is usually a self-resolving illness but can sometimes occur with symptoms like fever, anorexia, jaundice, nausea, vomiting, hepatomegaly, and abdominal pain[50]. Diagnosis is by serology, enzyme immunoassay, or RNA detection by PCR. Acute hepatitis E does not require treatment, however, if liver failure occurs then liver transplantation is an option[51]. Chronic hepatitis E can be treated with Ribavirin for 12 wk[52]. In pregnant women, HEV infection can lead to fulminant hepatic failure, which has a mortality rate of up to 30%[46]. Other complications include acute liver failure, chronic HEV infection, and neurological complications like Guillain-Barre syndrome, myelitis, and neuropathy to name a few[53]. Vaccines are not yet commercially available for prevention except in China[54].

BLOODY DIARRHEA FROM VIRAL GASTROENTERITIS

Cytomegalovirus

Cytomegalovirus (CMV) is a double-stranded DNA virus and a member of the Herpesviridae family with a prevalence ranging between 40%-100% in the adult population[55]. Latent phase reactivation occurs in immunocompromised individuals, including transplant recipients, patients on immunosuppressive agents, and those with inflammatory bowel disease (IBD) treated with steroids[56]. CMV is transmitted by contact with infectious body fluids like saliva, urine, respiratory droplets, sexual contact, blood transfusion, and solid organ transplants. Symptomology of CMV colitis could range from bloody diarrhea, abdominal pain, fever, weight loss, and lymphadenopathy to toxic megacolon. Few studies show that the severity of CMV is related to age and could cause Toxic megacolon and pan peritonitis in the elderly[57]. Although histology of tissue analysis is considered the gold standard for diagnosis, RT-PCR has the highest sensitivity and detection rate for infection. The most used antivirals are intravenous ganciclovir and valganciclovir. Foscarnet and cidofovir are used in ganciclovir-resistant cases. Some studies showed that although CMV is reactivated in IBD patients, it spontaneously disappears even without antiviral treatment[58,59]. Complications associated with CMV colitis may include ischemic colitis, perforation of the large bowel, toxic megacolon, and formation of pseudomembranous. Currently, there are no vaccines available for CMV. As per European Crohn's and Colitis Organization guidelines, if severe systemic CMV is detected, immunomodulators should be discontinued and screening for CMV is not routinely performed before starting immunomodulators in IBD[60].

Herpes simplex virus

Herpes simplex virus (HSV) is a double-stranded DNA virus belonging to the Human Herpesviridae family. HSV proctitis is the second most common sexually transmitted cause of infectious proctitis in homosexual males and could be caused by HSV-1 or HSV-2, but 70% of cases are due to HSV-2[61,62]. Prevalence of HSV-1 and HSV-2 have decreased over time linearly from 59.4 % & 18.0% in 1999-2000 to 48.1% & 12.1% in 2015-2016[63]. HSV is transmitted by intimate person-to-person contacts like men who have sex with men (MSM), unprotected receptive anal, and oral sex. Clinical symptoms of HSV proctitis include rectal bleeding, tenesmus, anorectal pain, and mucous discharge. The absence of external HSV lesions should not diminish suspicion of HSV infection as only 32% of men with HSV proctitis have external anal ulcerations[64,65]. Due to the high seropositivity for HSV worldwide, serological analysis plays a minor role in the diagnosis. PCR has been used to accurately diagnose and quantify the HSV DNA from clinical biopsy specimens[66]. Immunofluorescence staining of colonic specimens with HSV-type specific monoclonal antibodies against glycoproteins is highly specific to confirm the diagnosis. CDC recommends antiviral treatment with acyclovir, valacyclovir, or famciclovir in acute proctitis if the HSV infection is suspected or confirmed. Complications of HSV proctitis can result in symptoms such as constipation, severe anorectal pain, difficulty urinating, sacral paresthesia's, and diffuse ulcerations of the distal rectal mucosa. Currently, there are no vaccines approved for the prevention of HSV. Infectious proctitis must be considered before starting immunosuppressant therapy for presumed IBD, as the immunosuppressive medications may result in a lack of improvement or symptomatic worsening of infectious proctitis. HSV-induced anogenital ulcers will lead to a 1.5 to 7.0-fold increase in human immunodeficiency virus (HIV) transmission due to associated mucosal barrier breach, hence HIV screening is important. Individuals with acute proctitis along with HIV and/or painful perianal ulcers should receive presumptive treatment for anogenital HSV[67].

VIRAL GASTROINTESTINAL PATHOGENS ASSOCIATED WITH DIARRHEA AND OTHER GASTROINTESTINAL MANIFESTATIONS

Hepatitis A virus

Hepatitis A virus (HAV) is an RNA virus and a member of the Picornaviridae family. According to a report published in 2017, the incidence of acute hepatitis A was 170 million cases globally[68]. It is commonly transmitted through the fecal-oral route *via* contaminated food and water consumption. Transmission through sexual contact, person-to-person contact, and illicit drug use also exist in the literature[69]. Case fatality was higher in males, older than 50 years, and coexisting chronic liver disease raised the risk of developing fulminant hepatitis after an HAV infection[69]. From prior literature, > 70% of children under six years of age do not develop symptoms whereas > 70% of adults manifest symptoms[70]. Symptoms include fever, malaise, nausea, vomiting, abdominal discomfort, and jaundice. Physical examination findings include hepatomegaly and jaundice[70]. Hepatitis A generally follows a benign course, however chronic relapsing hepatitis for as long as 1 year is a possibility. Diagnosis is through serology by measuring the IgM antibody. IgG detection is useful when the question of immune status arises. Owing to its self-limited nature, this infection does not require treatment. According to the CDC, Hepatitis A vaccination is recommended for all infants. It is also recommended for those at substantial risk of exposure to hepatitis A infection, those at risk of

progressing to fulminant hepatitis, those experiencing homelessness, and HIV-infected persons.

Human immunodeficiency virus

Human immunodeficiency virus (HIV) is an RNA virus, belonging to the family Retroviridae. According to recent statistics, around 1.5 million individuals acquired HIV in 2021[71]. High-risk groups include gay and bisexual populations of all races and ethnicities, African Americans, injection drug users, and transgender populations[72]. It is transmitted *via* body fluids through sexual contact, needle sharing, breast milk, and perinatal transmission. In addition to the wide range of clinical manifestations, it is interesting to note how this virus affects the GI system. A specific term, "HIV enteropathy" has been coined to define the GI manifestations caused by this virus[73]. It causes alteration of epithelial ionic balances and enterocyte apoptosis resulting in inflammation, change in permeability, and malabsorption[73]. Histologically, villous atrophy, crypt hyperplasia, and epithelial hyperproliferation ensue. All of this culminates in causing diarrhea secondary to HIV enteropathy. HIV also leads to other GI effects by the virtue of its immunodeficiency, thus paving the way for opportunistic infections. Antiretroviral therapy (ART), especially protease inhibitors itself, can also cause diarrhea in HIV[74]. GI complications range from Esophageal disorders, gastric illnesses to colitis, enteritis, and anorectal disease. Most of these are caused by secondary/opportunistic bacterial, fungal, and viral infections and HIV-induced neoplasia of the GI tract. Also, the GI tract is a favorable site for HIV replication and GI CD4 destruction[75]. Pancreatic and hepatobiliary complications include pancreatitis, exocrine pancreatic insufficiency, hepatitis, and non-alcoholic fatty liver disease[76]. Diagnosis is fourth generation antigen-antibody assay followed by HIV 1/2 differentiation assay. Stool samples for ova and parasite examination should be done to identify the causative pathogen given the high chance of opportunistic infections like Cryptosporidium, Isospora, Giardia, *etc*[74]. If a cause cannot be identified yet, endoscopy with biopsy is an option[74]. Highly ART (HAART) causes the reconstitution of peripheral circulating plasma CD4 cells, but studies have found that it is not successful in the replenishment of GI CD4 cells[77].

Hepatitis B and D virus

Hepatitis B virus (HBV) is a partially double-stranded DNA virus and belongs to the family of Hepadnaviridae. According to the CDC statistics, globally, 296 million people are infected with Hepatitis B. High-risk groups include veterans, healthcare professionals, MSM, injection drug users, persons with HIV, hepatitis C virus (HCV) co-infection[78]. hepatitis B can be transmitted perinatally, through sexual contact, percutaneous or person-to-person contact with infected body fluids[79]. The incubation period ranges from 1 mo to 4 mo. The acute phase of hepatitis B infection presents as a serum-sickness-like illness characterized by fever, rash, and arthralgia followed by jaundice, nausea and vomiting, and other constitutional symptoms[79]. It causes elevation of alanine aminotransferase (ALT) more than aspartate aminotransferase (AST) and bilirubin. Diagnosis is by serum viral biomarkers[79]. It is known that Hepatitis B is implicated in the causation of Hepatitis and its plethora of clinical manifestations including hepatocellular carcinoma (HCC). HCC contributes to 80% of global liver cancers in 2018[80]. Pathogenesis of HCC in hepatitis B is: (1) Direct-due to the oncogenic viral protein activating proto-oncogenes, transcriptional pathways (MAP kinase and JAK/STAT), and inhibition of tumor suppressor genes (p53); (2) chronic inflammation, cirrhosis, and regeneration[81,82]. It is also worth noting the other GI disorders caused by this virus. According to a cumulative analysis performed by Yang *et al*[83] involving 7027546 individuals across 13 studies-10 studies reported data on hepatitis and gastric cancer, and it was found that the risk of gastric cancer was 26 times higher in the hepatitis B population[83] (pooled HR, 1.26; 95%CI, 1.08-1.47; P = 0.003). This can be attributed to chronic inflammation, tumorigenesis, and alteration of the tumor suppression process due to oncogenic viral proteins [84,85]. Another study observed that Hepatitis B is associated with gut microbiota disturbance, especially in the cirrhosis population[86]. Acute HBV is self-limited and does not require treatment. Special cases like acute liver failure, complicated course (prolonged PT and marked elevation of jaundice are treated[87]. Treatment of chronic hepatitis B depends on factors like the presence or absence of cirrhosis, ALT level, and HBV DNA level. Therapy includes drugs like Tenofovir, Entecavir, and Interferon[87]. Complications of acute hepatitis B are mainly due to immune complex reactions occurring in various parts of the body manifesting as glomerulonephritis, polyarteritis nodosa, cryoglobulinemia[88], *etc.* Sequelae of chronic hepatitis B are well known including cirrhosis and HCC. Hepatitis B prevention is achieved with recombinant vaccines that require either three or two doses. Combined vaccination, along with diphtheria, pertussis, tetanus, and hepatitis A is also in use currently in the United States.

Hepatitis D virus (HDV) is a defective RNA virus requiring the presence of the Hepatitis B virus for its replication and assembly of virions. It belongs to the Kolmioviridae family. The global disease burden of hepatitis D/hepatitis B co-infection is 62-72 million[89]. HDV requires HBV to replicate and therefore the HBV population is the target group for this infection. The mode of transmission is the same as HBV infection. Hepatitis D infection can occur as a co-infection with hepatitis B occurring simultaneously which usually leads to spontaneous resolution[90]. On the other hand, superinfection in already infected HBV persons usually leads to conversion to chronic hepatitis D infection which is considered a severe form of chronic hepatitis. Symptoms of acute hepatitis D are indistinguishable from

other forms of viral hepatitis. However, superinfection with HBV can lead to fulminant liver failure[91]. In addition, the risk of progression to cirrhosis is 3 times higher in superinfection than in infection with HBV alone[92]. Diagnosis is through the detection of IgM and IgG Anti HDV antibodies and confirmed by testing for HDV RNA[93]. The current treatment strategy utilizes Pegylated interferon for at least 1 year[94]. However, several emerging therapies like myrcludex B and Lonafarnib are gaining popularity in the treatment of hepatitis D.

HCV

HCV is a hepatotropic, single-stranded RNA virus belonging to the family of Flaviviridae. The prevalence of Hepatitis C is 0.5%-2.5% with the highest being in the eastern Mediterranean region and Europe[95]. It was recorded that 399000 individuals died in 2016 due to complications from chronic hepatitis C[95]. Injection drug users, HIV, healthcare professionals, prior recipients of blood products (before 1992), and hemodialysis patients are at risk of acquiring HCV[96]. Modes of transmission include injection drug use, blood transfusion, sexual contact, and perinatal transmission[97]. Acute hepatitis C infection is usually asymptomatic and can consist of symptoms like fever, abdominal pain, and jaundice[97]. Only 15%-20% resolve completely whereas the remaining percentage of infected patients go on to have chronic hepatitis C[97]. Chronic hepatitis C is characterized by the presence of HCV RNA for more than 6 mo. Sequelae of chronic hepatitis C are liver fibrosis, Cirrhosis, and Hepatocellular Carcinoma. A decision analytical model done by Chen *et al*[98] predicted that the cumulative incidence of HCC among HCV-infected persons would be 583000 cases between 2012 to 2040. Several extrahepatic manifestations affect the quality of life in chronic hepatitis C like mixed cryoglobulinemia, glomerulonephritis, skin disease (porphyria cutanea tarda and lichen planus), thyroid disease (Hashimoto's disease and Grave's disease) to name a few[99]. Diagnosis is made by serology either by the presence of HCV RNA or anti-HCV antibody[100]. Guidelines suggest treatment with direct-acting antiviral agents (DAA) after acute infection to prevent progression to chronic infection and due to the high likelihood of loss to follow-up to test for spontaneous clearance of the virus[101]. Chronic hepatitis C is treated with DAA regimens based on the genotype and also the presence of advanced liver disease. Some of the pan-genotype regimens include Sofosbuvir and velpatasvir for 12 wk and glecaprevir and pibrentasvir for 8 wk[102]. Vaccination for hepatitis C is under development and prevention of hepatitis C solely depends upon the prevention of high-risk behavior like injection drug use and adopting safe sexual practices.

Varicella zoster virus

Varicella-zoster virus (VZV) is a double-stranded enveloped DNA virus belonging to the α-herpesviruses subfamily. Adults, young children, and immunocompromised are at risk of developing the severe disease with primary varicella infection[103]. According to the CDC report, 4 million cases occurred annually in the United States during the pre-vaccine era. After the introduction of vaccines, incidence declined by around 97%[104]. Transmission is through droplets, aerosols, and direct contact with respiratory secretions or zoster lesions[105]. Reactivation of latent VZV from dorsal root ganglia leads to herpes zoster (shingles), which presents as localized cutaneous eruption associated with neuralgic pain. GI VZV lesions could present as multiple erosions occurring in the stomach, duodenum, and small and large intestines[106]. Skin findings often precede visceral involvement. In a study, 42 of 131 patients with herpes zoster [not specified as bone marrow transplant (BMT) or malignancy patients] progressed to visceral involvement[107]. The mortality rate from GI VZV ranges from 28.6% to 50.0% in BMT recipients despite antiviral therapy[108]. GI manifestations of herpes zoster are extremely rare. Constipation secondary to motility issues (visceral neuropathy) has been reported in some case reports [106,109]. Skin findings and concomitant GI symptoms should raise suspicion in predisposed patients. Elevated liver enzymes and abnormal imaging findings also aid in diagnosis. Diagnosis can be confirmed through immunohistochemical staining of biopsy specimens VZV. Although shingles is a clinical diagnosis, PCR (highly sensitive) and immunoassays are used for the diagnosis, especially in atypical presentations[110]. Treatment options include IV Acyclovir, valacyclovir, famciclovir, and brivudine. If clinical resistance to Acyclovir is suspected, foscarnet could be used. Complications of primary varicella infection are bacterial infections of the skin and soft tissues, pneumonia, and cerebellar ataxia[111]. Reactivation of VZV (Herpes zoster) is complicated by chronic pain, encephalitis, post-herpetic neuralgia, myelopathy, *etc*[112]. Varivax, a two-dose vaccine, is licensed for children above 12 mo of age to protect against chicken pox. Shingrix is an FDA-approved recombinant vaccine to reduce the risk of shingles in people aged 50 years or more.

Epstein-Barr virus

Epstein-Barr virus (EBV) is a double-stranded DNA virus belonging to the Herpesviridae family. It is estimated that more than 90% of the worldwide population has been infected with EBV[113]. It is associated with a wide range of clinical manifestations, the most common one being infectious mononucleosis (IM), prevalent among teens and adults[114]. It is spread through saliva and presents with fever, malaise, sore throat, and lymphadenopathy[114]. Chronic active EBV (CAEBV) is a condition where IM symptoms persist for more than 3 mo and is commonly seen in Asia[115]. EBV has also been

implicated in the pathogenesis of multiple sclerosis and rheumatoid arthritis[115]. Also, chronic EBV has been linked with gastritis, enteritis, and esophageal disorders, oral hairy cell leukoplakia[116-119]. A separate clinical subset of diseases EBV has been strongly associated with is lymphoproliferative cancers like Burkitt's Lymphoma, Hodgkin's lymphoma, Non-Hodgkin's lymphoma, and post-transplant lymphoproliferative disorder. Besides lymphoproliferative cancers, EBV has been implicated in causing other cancers like gastric cancer, nasopharyngeal cancers, and breast cancer. Some key features of EBV-associated gastric cancers are they can occur in the gastric fundus, cardia, and body, unlike non-EBV-associated gastric cancers which happen in the antrum. EBV-associated cancer starts as a lesser fibrotic nodular ulcer compared to the non-EBV type[119]. Microscopically, EBV-associated gastric cancer shows a monoclonal proliferation infected latently with EBV, suggesting the role of the virus in the early stages of tumorigenesis[119]. IBD patients on immunosuppressants like thiopurines have a high incidence of EBV and some studies also showed that patients taking these medications also have a higher incidence of lymphoproliferative disorders than those who are not on treatment for IBD [120,121]. EBV can be diagnosed by the detection of antibodies against various antigens like viral capsid antigens, EBNA; enzyme immunoassays, Western blot, PCR, heterophile antibody agglutination, *etc* [122]. There is no targeted therapy for EBV. Symptomatic treatment has been a cornerstone for the treatment of EBV. Several anti-viral drugs including acyclovir, and cidofovir have been tried with good invitro activity and with no clinical benefit[123].

Human herpesvirus 8

Human herpesvirus 8 (HHV 8) is a double-stranded DNA virus belonging to the Human Gamma herpesvirus family. It is also commonly known as Kaposi Sarcoma-associated herpesvirus due to the disease it causes, Kaposi's Sarcoma (KS). It is common in HIV, MSM, Mediterranean, Ashkenazi jews, and sub-Saharan African populations[124,125]. According to seroprevalence, Uganda has the highest seroprevalence worldwide where KS is endemic. No more than 6% seroprevalence has been reported in the United States[126]. KS is a low-grade vascular tumor that involves mucocutaneous sites and visceral locations, predominantly the respiratory and GI systems[125]. GI Kaposi sarcoma is usually asymptomatic but on progression, can present as abdominal pain, nausea, vomiting, and GI bleeding. Diagnosis is through endoscopy and biopsy[127]. It is most common in the stomach and small intestine and endoscopically appears as a maculopapular lesion to a nodular or polypoid mass which can sometimes bleed on touch[127]. In HIV Kaposi, HAART is the mainstay of treatment with or without chemotherapy. In classic Kaposi's sarcoma, treatment options include local therapy with radiation or intralesional chemotherapy; systemic chemotherapy with liposomal doxorubicin or paclitaxel[124]. Besides KS, HHV 8 is implicated in the causation of lymphoproliferative disorders like primary effusion lymphoma and multicentric Castleman's disease[128].

Human papillomavirus

Human papillomavirus (HPV) is a double-stranded DNA virus belonging to the Papillomaviridae family. It is estimated that around 13 million persons acquired HPV infection in 2018 in the United States and over 77 million had a prevalent infection during the same year[129]. It is primarily a sexually transmitted disease (vaginal, anal, and oro-genital) but can also be transmitted through skin-to-skin contact and vertical transmission[130]. High-risk sexual behaviors, multiple sexual partners, and previous history of STDs increase the risk of HPV acquisition. Clinical manifestations of HPV include cutaneous warts, anogenital warts, and respiratory papilloma's caused by HPV 6,11 *etc* genotypes along with cancer precursor lesions (intraepithelial neoplasia's) and cancers caused by HPV 16,18 genotypes. HPV is an oncogenic virus and has been associated with a multitude of cancers including GI cancers like esophageal cancer, stomach cancer, colorectal cancer, anal cancer, and liver cancer[131]. The other cancers to which it has been linked are cervical, penile, vulvar, vaginal, and oropharyngeal cancers [132]. HPV causes cervical cancer near the transformation zone where there is a transitional/transformational zone (squamocolumnar junction). In the same fashion, HPV also causes cancerous lesions in the anal region because of the presence of a squamocolumnar junction which is a site of multipotent embryonic cells. However, the incidence rates of cervical cancer and anal cancer are much different (17:1). Colposcopy, Biopsy, HPV DNA detection, PCR, and pap smear are some of the methods by which HPV can be diagnosed[133]. Treatment depends on the type of disease. Warts can be treated with topical medications like salicylate, imiquimod, trichloroacetic acid, cryosurgery, and electrocautery. Precancerous and cancerous lesions require detailed workup and surgical excision of lesions[134]. Current FDA-approved vaccines are Bivalent (for types 16 and 18), quadrivalent (for types 6, 11, 16, and 18), 9 valent (for types 6, 11, 16, 18, 31, 33, 45, 52, and 58) protect against genital warts, precancerous lesions of vulva, cervix and anus, oropharyngeal cancers and are approved for males and females aged 9-45.

CONCLUSION

After conducting a comprehensive review of the literature on viruses and the GI tract, these pathogens

play a significant role in both acute and chronic GI diseases. While many viruses can cause mild symptoms, such as diarrhea and vomiting, some can lead to severe and even life-threatening conditions. The importance of early detection and appropriate management of viral gastroenteritis cannot be overstated.

Several studies have highlighted the need for better prevention and control measures, including improved hygiene practices and the development of effective vaccines. It is crucial to continue researching the relationship between viruses and the GI tract to better understand how these pathogens operate and to develop more targeted treatments.

Overall, this review provides clinicians with a differential diagnosis when they encounter patients with GI manifestations. It emphasizes the importance of recognizing the different presentations of viruses on the GI tract and understanding the prevention, diagnosis, and treatment of these illnesses.

FOOTNOTES

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REVIEW

Impacts of SARS-CoV-2 on diabetes mellitus: A pre and post pandemic evaluation

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic caused by the novel beta coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) crippled the whole world and has resulted in large number of morbidity and mortality. The origin of the SARS-CoV-2 is still disputed. The risk of infection with SARS-CoV-2 is dependent on several risk factors as observed in many studies. The severity of the disease depends on many factors including the viral strain, host immunogenetics, environmental factors, host genetics, host nutritional status and presence of comorbidities like hypertension, diabetes, Chronic Obstructive Pulmonary Disease, cardiovascular disease, renal impairment. Diabetes is a metabolic disorder mainly characterized by hyperglycemia. Diabetic individuals are intrinsically prone to infections. SARS-CoV-2 infection in patients with diabetes result in β-cell damage and cytokine storm. Damage to the cells impairs the equilibrium of glucose, leading to hyperglycemia. The ensuing cytokine storm causes insulin resistance, especially in the muscles and liver, which also causes a hyperglycemic state. All of these increase the severity of COVID-19. Genetics also play pivotal role in disease pathogenesis. This review article focuses from the probable sources of coronaviruses and SARS-CoV-2 to its impacts on individuals with diabetes and host genetics in pre- and post-pandemic era.

Key Words: Coronavirus; SARS-CoV-2; Diabetes; MERS; SARS; Single nucleotide polymorphism

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Core Tip: The coronavirus disease 2019 (COVID-19) pandemic caused by the novel beta coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) crippled the whole world and has resulted in large number of morbidity and mortality. The origin of the SARS-CoV-2 is still disputed. The risk of infection with SARS-CoV-2 is dependent on several risk factors as observed in many studies. The severity of the disease depends on many factors including the viral strain, host immunogenetics, environmental factors, host genetics, host nutritional status and presence of comorbidities like hypertension, diabetes, Chronic Obstructive Pulmonary Disease, cardiovascular disease, renal impairment. Diabetes is a metabolic disorder mainly characterized by hyperglycemia. Diabetic individuals are intrinsically prone to infections. SARS-CoV-2 infection in patients with diabetes result in β-cell damage and cytokine storm. Damage to the cells impairs the equilibrium of glucose, leading to hyperglycemia. The ensuing cytokine storm causes insulin resistance, especially in the muscles and liver, which also causes a hyperglycemic state. All of these increase the severity of COVID-19. Genetics also play pivotal role in disease pathogenesis. This review article focuses from the probable sources of coronaviruses and SARS-CoV-2 to its impacts on individuals with diabetes and host genetics in pre- and post-pandemic era.

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DIABETES AND PRE PANDEMIC ERA

The term “diabetes” first appeared in a medical text book around 1425 and was first suggested by a Greek scientist, Araetus of Cappodocia (81-133AD)[1]. Thomas Willis added the word "mellitus" (meaning "sweet taste of urine") to the word diabetes in 1675. Diabetes was referred to as Madhumeha in ancient Indian medicine[1,2]. In 1776, a British physician named Matthew Dobson identified excess sugar in urine and blood as the cause of their sweet taste, and glucose was identified as the responsible sugar[1,2]. The experimental creation of diabetes in 1889 in experimentally pancreatectomized dogs eventually confirmed that the pancreas is the causative organ of the disease followed by the isolation of insulin in 1922, which finally established that diabetes is an endocrine disorder due to deficiency of insulin[2-4].

Diabetes mellitus has been recognized as one of the major health concerns and is considered a global epidemic affecting 382 million people worldwide. According to the prediction of the World Health Organization, diabetes will be the seventh leading cause of death by 2030[5]. Diabetes is a metabolic disorder caused by hyperglycemia and characterized by polyuria, polyphagia, polydipsia, and weight loss. Diabetes mellitus causes macrovascular and microvascular complications. Macrovascular complications include coronary heart disease, cardiomyopathy, arrhythmias and sudden death, cerebrovascular disease and peripheral artery disease. Myocardial infarction, stroke, and peripheral artery disease are more prevalent in individuals with diabetes mellitus. Microvascular complications include retinopathy, nephropathy, and neuropathy. These complications are the major contributing factors to the increasing mortality rate in patients with diabetes and an estimated 4 million individuals are dying each year due to diabetes related complications.

American Diabetes Association classified diabetes into four categories: Type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM), and diabetes induced by or related to particular specific illnesses, pathologies, and/or syndromes are the four primary forms or categories of diabetes, respectively. T1DM, often referred to as type 1A diabetes mellitus (DM), insulin-dependent diabetes mellitus (IDDM), or juvenile-onset diabetes, accounts for around 5%-10% of all diabetes cases. It is an autoimmune condition marked by the T-cell-mediated apoptosis of pancreatic beta-cells, which causes an insulin shortage and ultimately leads to hyperglycemia. T2DM, also known as non-insulin-dependent diabetes mellitus accounts for 90%-95% of all cases of diabetes. This kind of diabetes is characterized by two main insulin-related abnormalities: Insulin resistance and β-cell dysfunction. Any degree of glucose intolerance or diabetes, typically discovered in the second or third trimester of pregnancy, is referred to as GDM. Other than T1DM, T2DM, and GDM, other types of diabetes although in smaller percentages relative to the overall diabetic incidence scenario, has been found to be linked to a number of other illnesses, including several pathologies[6].

Endocrinopathies, exocrine pancreatic diseases, diabetes caused by monogenic deficiencies in β-cell function, and diabetes caused by genetic abnormalities in insulin action are the most common types of diabetes. Type 1 diabetes is a chronic autoimmune disease that has both hereditary and environmental causes. In those who have a genetic predisposition to developing autoimmunity, viruses may affect the susceptibility of the infectious disease and trigger it. The most frequently studied viruses in relation to type 1 diabetes are enteroviruses. Pancreatic islet autoimmunity and the onset of type 1 diabetes clinical

symptoms have also been linked to respiratory viral infections. In animal models, the influenza virus can infect human pancreatic cell lines and result in pancreatitis and hyperglycemia. In case-report studies, influenza A virus infection has also been linked to acute pancreatitis and type 1 diabetes. There may be a connection between pandemic influenza and type 1 diabetes, according to two small retrospective investigations. These revealed a parallel rise in type 1 diabetes among kids throughout the pandemic influenza timeframe. It normally takes several years from the induction of islet autoimmunity to the clinical presentation of type 1 diabetes, hence studies with longer follow-up following influenza are necessary to clarify how pandemic influenza contributes to the development of diabetes.

Diabetes patients are up to six times more likely than healthy people to require hospitalization for influenza virus or flu-like infections, and they are also more likely to experience infection related complications. Diabetic individuals, especially those with uncontrolled diabetes, are more prone to fungal infections[7]. Community-based pneumonia was more frequent in those diagnosed with diabetes mellitus[8]. Increased mortality risk from cardiovascular disease, chronic lower respiratory illnesses, influenza and pneumonia, and kidney disease is linked to baseline diagnosed diabetes[9]. A regulated immune response that leads to more severe and prolonged lung pathology is most likely the cause of the higher disease severity seen in mice with REMS and co morbid type 2 diabetes[10]. According to a systematic analysis of 637 Middle East respiratory syndrome (MERS)-CoV cases, 50% of the patients had both diabetes and hypertension. Obesity was prevalent in 16% of cases, while cardiac problems were present in 30%[11].

SARS CORONAVIRUS AND THE PRE- PANDEMIC ERA

There are similarities between many epidemics and industrial revolutions that have occurred throughout history. No other virus, bacteria, or germ has ever produced as many pandemics and as many deaths on Earth in as little time as plagues and flu. Plague is a disease caused by a bacterium called Yersinia pestis, which is mainly transmitted by rats and one type of fly. History shows that the first plague occurred between 165 and 180 AD (also known as the Antonine Plague that brought the Roman Empire to the brink of destruction) and caused the deaths of 500000 people. Plague outbreaks followed in 541-542 AD, 1347-1351 AD, 1665 AD and 1629-1631 AD. At this time, the word “industrial” revolution did not appear in the world. But history tells us that the plague that occurred in 1885 between the first and second industrial revolutions caused the death of more than a million people in China and India alone. During the Second Industrial Revolution in 1889-1890 and 1918-1919, respectively, the Russian flu and the Spanish flu struck, which took the lives of about 60 million people in the world. During the Third Industrial Revolution, Asian flu (1957-1958), Hong Kong flu (1968-1970), and AIDS (still ongoing since 1981) killed over 40 million people. Among these, flu-like diseases are caused by different types of influenza viruses that belong to the same genus but differ in the structure of their genetic material. The genetic material of the influenza, swine flu viruses is formed by ribonucleic acid, or RNA.

Different types of wildlife frequently carry diseases or disease agents that are harmful, and in some cases fatal, to humans. Recent studies have shown that 60% of the infectious diseases that are currently occurring are zoonotic, that is, the bacteria responsible for these diseases spread from animals to humans. Although these bacteria are common in animals, they can also infect humans. Also 70% of infectious diseases originate from wildlife[12].

The study of Corman *et al*[13] revealed that bats are the natural host of the coronavirus. Even the age of corona virus found in bats is much longer than that of coronavirus found in other animals. This suggests that the virus has overcome the barriers that normally exist to spread from one species to another and has now adopted humans as its home[14]. Bats are also thought to be the source of two other strains HCoV-NL63 and HCoV-HKU1[15]. Every organism has a natural reservoir. The more we disturb nature, the more likely it is to deplete natural resources. As a result, the microbes are in crisis of existence and to adapt to the changing environment, they cause rapid change or mutation. The significant natural reservoirs of the coronavirus are bats, camels and palm civets. Because of its crown like shape, it is called the "coronavirus". Before making devastating impacts, the human race has always received signs of warnings for larger damage. For example, SARS in 2002-2003, swine flu (2009-2010) in 2009-2010 and MERS, MERS from 2015 to the present.

Coronavirus can be generally divided into four genera - alphacoronavirus, beta coronavirus, gamma and delta. Among these, the beta group is further divided into four groups – A, B, C and D. Human strains of coronaviruses include HCoV 229E (HCoV 229E) and HCoV NL63 of the alpha genus, and HCoV OC43 and HKU1 of the beta genus. Severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) are also members of the beta genus. A study in 2003 found that the genome of SARS-CoV-1 in humans was 99.8% identical to that of SARS-CoV in palm civets. Later, the genome of human SARS-CoV-1 was found to be approximately 87%-92% similar to that of SARS-CoV recovered from Chinese bats. From this, it is assumed that the natural source of this virus is the Chinese bat. The virus is transmitted to humans using the palm civet as an intermediate vector.

Since the outbreak of MERS-CoV, scientists have begun more research on bat-borne coronaviruses. In line with this, a study in Saudi Arabia found that the genome sequence of human MERS-CoV is nearly 100% identical to a virus found in bats called Taphozous perforates. In addition, some preliminary studies have found viruses similar to this virus in several species of bats. The scientists then sequenced the replication gene of MERS-CoV and found that the virus resembled the HKU4 coronavirus of Tylonycteris bats. However, a 2013 study found that MERS-CoV is actually a member of the beta-coronavirus family and that camels are intermediate carriers of the virus.

NOVEL CORONAVIRUS OR SARS-COV-2

The Coronaviruses are enveloped virus. It is a positive sense, single-stranded RNA composed of about 29000 nucleotides. 229E (HCoV-229E) virus is a close relative of the currently prevalent CoV virus in humans due to several conformations. Their habitat was also known to be in the hipposideridae family of African bats. It is hypothesized that they may have used dromedaries as intermediate habitats between bats and humans[16].

The HCoV virus has also been found to be related to viruses in other animals. For example, HCV-OC43 has been shown to be highly homologous to mouse hepatitis virus and bovine respiratory coronavirus[13]. The habitats of these two viruses are rats and cows respectively. An enzyme called hemagglutinin esterase is found in all of them.

Pangolin is also another natural reservoir of coronavirus. Analysis of the Genome Sequence of the currently alarming virus has shown that some parts of the RNA of Novel Coronavirus 2019 or COVID-19 came from bats and some parts came from Pangolin, and this recombination process took place in a third animal. We are destroying these animals for food and medicine.

The genome of SARS-CoV-2 is similar to that of other beta coronaviruses. Most of the proteins that SARS-CoV-2 encodes are similar in length to those that SARS-CoV encodes[17]. From 5′ to 3′, the genomic structure contains leader sequence, ORF1/ab, Spike (S), ORF3a, Envelope (E), Membrane (M), ORF6a, ORF7a, ORF7b, ORF8, Nucleocapsid (N), ORF10 and lacks the hemagglutinin-esterase gene which is found in some β-CoVs. ORF1a/b is made up of 16 non-structural proteins (nsp1–16) and accounts for almost two-thirds of SARS-CoV-2 RNA. The replicase gene encodes a large polyprotein (pp1ab), which is involved in transcription and virus replication. It is fragmented by proteolysis into 16 non-structural proteins. The remaining one-third of the genome, at the 3′-terminus, contains ORFs that code for structural and auxiliary proteins of SARS-CoV-2[18]. With the exception of the S protein, which is different, the structural proteins (E, M, and N) of SARS-CoV-2 share around 90% of their amino acid composition[19,20]. The majority of SARS-CoV-2 non-structural proteins share more than 85% of their amino acid sequences with SARS-CoV[17].

ZOONOSIS OF CORONAVIRUSES

Endemic coronaviruses have animal origins: HCoV-NL63 and HCoV-229E are speculated to have originate in bats whereas; HCoV-OC43 and HKU1 are likely to have originated in rodents[21,22]. Bats are also considered as the natural reservoir of SARS-CoV, MERS-CoV and SARS-CoV-2[16,20,23]. Masked palm civet and dromedary camel are the intermediate hosts of SARS-CoV and MERS-CoV, respectively[23]. However, the intermediate host of SARS-CoV-2 is not clear till date. Pangolin, snakes, minks, turtles, ferret and companion animals are possible candidates for the intermediate hosts of SARS-CoV-2[24] (Figure 1).

Cohorts from The United States, Germany, Netherlands, Singapore and the United Kingdom that 20% to 50% individuals not exposed to SARS-CoV-2 previously had T cell activity against peptides that match the sequences of the SARS-CoV-2[25-29]. The T cell activity was mostly due to CD4+ T cells. This might be due to the presence of preexisting memory against endemic coronaviruses which share sequence homology with SARS-CoV-2.

COMORBIDITIES AND COVID-19

Despite the fact that risks are generally higher in men and rise with age, there is now compelling evidence that those who have a number of medical conditions—including chronic kidney disease, diabetes, lung and liver disease, cardiovascular disease, obesity, immunodeficiency, certain disabilities, and mental health conditions—are also at higher risk[30].

People with complex diabetes, obesity, and psychological disorders are at the highest risk (relative risk of about 1.3 compared to those without these illnesses), whereas those with cardiovascular disease are at a lower risk (relative risk roughly 1.1)[31]. The evidence is inconclusive for asthma, hypertension, and viral hepatitis, while it is scarcer for other illnesses like obesity, sickle cell disease, and substance

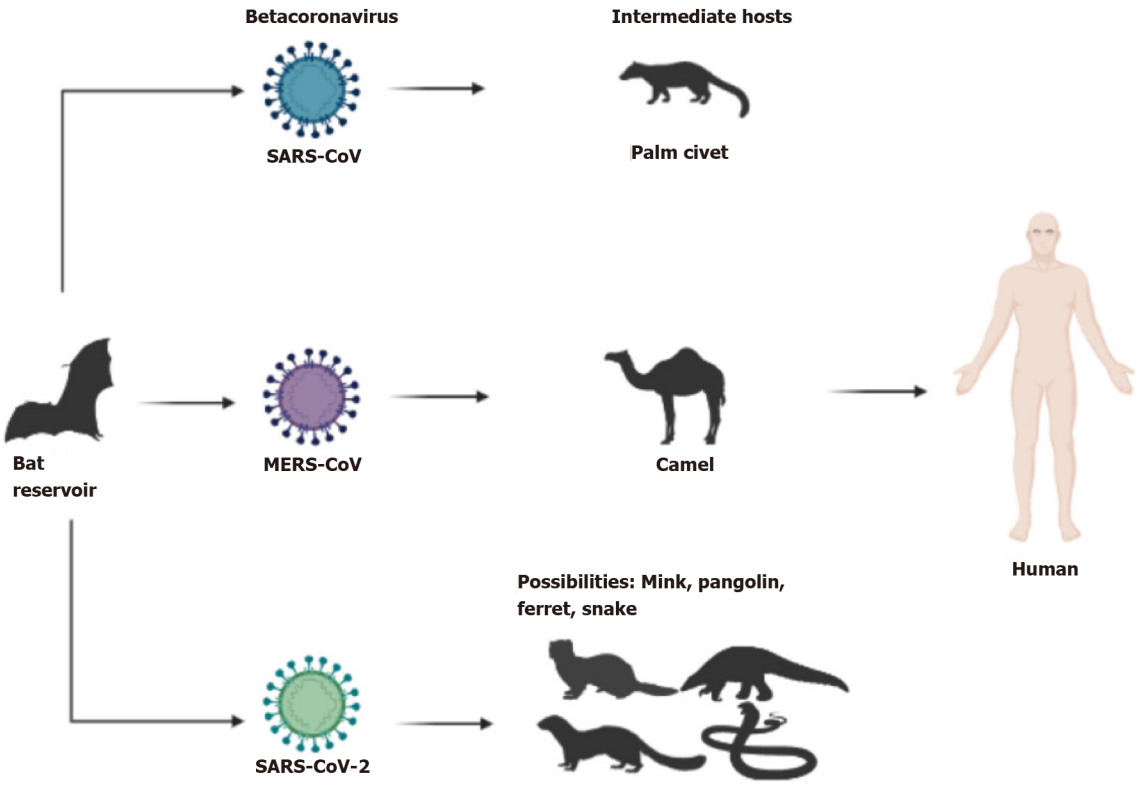


Figure 1 Possible intermediate hosts of severe acute respiratory syndrome coronavirus 2. Bat is considered as the natural reservoir of the pandemic-causing beta coronaviruses: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV. The intermediate hosts of SARS-CoV and MERS-CoV are palm civets and dromedary camels, respectively. Although mink, pangolin, ferret and snake have been considered as the intermediate hosts of SARS-CoV-2, however the issue is still unresolved. The figure was created using BioRender (https://biorender.com/).

use disorders. Inflammatory and hormonal routes, as well as social factors like being in a crowded or institutionalized environment, are proposed as possible disease susceptibility and severity processes, albeit the precise mechanisms remain unknown[32,33].

Based on the frequency of chronic diseases, it is projected that one in five people globally are at an increased risk of negative COVID-19 outcomes. Along with age, the risk rises when there are more underlying conditions[34]. Ages 50 to 64 have a fourfold rise in mortality risk compared to those under 40, and 85 and older see a more than tenfold increase[35]. Similar to this, those with one comorbidity or more than 10 comorbidities had a 1.5- and 3.8-times higher risk of dying than those without any underlying diseases[31]. To assist clinical judgments, a number of risk score calculators have been developed using these data[36,37]. Previous research has shown that individuals with H7N9 infection have a 3.4-fold higher chance of having acute respiratory distress syndrome when any comorbidity is present. Similar to the influenza virus, the coronavirus causing SARS-CoV and the MERS-CoV, Covid-19 more easily predisposes susceptible patients to respiratory failure and mortality[38].

A report consisting of 72314 cases in China, case fatality rate was observed to be increase in individuals with preexisting comorbidities like cardiovascular disease (10.5%), diabetes (7.3%), chronic respiratory disease (6.3%), hypertension (6.0%) and cancer (5.6%)[39]. In a study, 399 instances (25.1%) out of the 1590 reported having at least one comorbidity. The prevalence of specific comorbidities included hepatitis B infections (28; 1.8%), chronic obstructive pulmonary disease (24; 1.5%), chronic kidney disease (21; 1.3%), malignancy (18; 1.1%), and immunodeficiency (3; 0.2%). Hypertension (269; 16.9%), other cardiovascular diseases (53.7%), cerebrovascular diseases (30; 1.9%), diabetes (130; 8.2%), and diabetes-related complications were also common. None of the cases had asthma diagnosed by a doctor. In severe instances *vs* non-severe cases, at least one comorbidity was more frequently observed (32.8% *vs* 10.3%). Patients with at least one comorbidity tended to be older (mean: 60.8 *vs* 44.8 years), more likely to experience shortness of breath (41.4% *vs* 17.8%), nausea, or vomiting (10.4% against 4.3%), and to present abnormally on chest X-rays (29.2% *vs* 15.1%)[38].

DIABETES AND COVID-19

The world has experienced the devastating impact of infectious organism novel coronavirus or SARS-CoV-2 since it's inception in December 2019. Physicians faced enormous challenges to battle against the COVID-19 related complications especially determining or selecting the way of managing the disease *i.e.*, which group of individuals are at higher risk of worst severity[40]. Epidemiological evidences supported that comorbidities have added extra burden to the miseries of pandemic. Among the comorbidities, diabetes took the lead. Further, individuals with diabetes had greater risk of sufferings from the complications caused by this virus. The ongoing COVID-19 pandemic has once again emphasized the importance of preventing and managing type 2 diabetes. Complications even worsen in individuals with both heart disease and diabetes or macrovascular complications due to chronic diabetes.

Diabetic individuals are more prone to both primary and secondary infections caused by bacteria, viruses, fungus and as disease progresses, severity of the disease intensifies compared to their nondiabetic counterparts[10,41]. Obesity and poor control of blood glucose has been found to be directly linked to the clinical outcomes of COVID-19 and poor prognosis followed by increased mortality[42-45]. Zhu *et al*[45] in a cohort study comprised of 7337 confirmed COVID-19 cases demonstrated that the occurrence of ARDS (16.9% *vs* 7.2%), acute heart injury (7.3% *vs* 3.0%), acute kidney injury (3.9% *vs* 0.8%), septic shock (3.8% *vs* 1.0%), and DIC (0.5% *vs* 0.2%) were in individuals with T2D compared to the non-diabetic group. SARS-CoV-2 infects multiorgan system and pancreas is no exception as this organ also expresses angiotensin converting enzyme 2[46]. Previous findings reported presence of angiotensin converting enzyme 2 (ACE2) receptor in the pancreatic beta cells for SARS-CoV-1 virus that acts as the viral entry point leading to the destruction of β-cells and insulin insufficiency causes hyperglycemia[47]. Studies reported morphological, translational and functional modification of pancreas followed by impaired insulin secretion as SARS-CoV-2 is able to successfully infect different cell types of both exocrine and endocrine system which include pancreas[48,49]. SARS-CoV-2 infected patients with poor control of blood sugar had high mortality rate compared to those with well controlled blood glucose. Further investigation revealed that even individuals suffering from COVID-19 disease with a glucose range of 3.9 to 10.0 mmol/L had a lower mortality rate than that of their counterparts with blood glucose levels above 10.0 mmol/L[44]. Moreover, COVID-19 patients with poorly controlled blood glucose had to extend their hospital stay[44]. Thus, better management of blood glucose results in improved outcome in patients with multiple organ damage and reduced the mortality rate in COVID-19 patients[45,50].

In an investigation of two major health-care datasets, US CDC researchers discovered that COVID-19 was associated with a higher risk of diabetes than pre-pandemic acute respiratory infections and that non-SARS-CoV-2 respiratory infections were not. In the post-acute phase of COVID-19, those under the age of 18 who had SARS-CoV-2 infection had a higher probability of being diagnosed with diabetes than non-infected controls. Despite this, the study was unable to differentiate between type 1 and type 2 diabetes[51].

A Scottish study of individuals under the age of 35 found an overall 20% rise in the incidence of type 1 diabetes during the pandemic and an elevated risk of the disease within the first 30 days following SARS-CoV-2 infection, but not afterward[52]. Another study of 428650 individuals with COVID-19 and 428650 matched controls revealed a net increase in the incidence of diabetes in the first four weeks following COVID-19. This increase persisted from five to twelve weeks but not from thirteen to fifty-two weeks. The participants in this study had a median age of 35[53].

CYTOKINE STORM, COVID-19 AND DIABETES OR DIABETES, COVID-19 AND INFLAMMATION

Several advances have been made in understanding the pathophysiology of type 2 diabetes. Inflammation plays an important role in the pathogenesis of type 2 diabetes as it mediates hypoxia and cell death of adipose tissue, activation of important factors in signal transduction *i.e.*, nuclear factor-κB (NF-κB) and JUN N-terminal kinase, interleukin-1β and recruitment of immune cells. Factors associated with innate immune response are found to be in the circulation, insulin-sensitive tissues and pancreatic islets in type 2 diabetes. Obesity, one of the major causes of developing insulin resistance followed by type 2 diabetes, leads to elevated levels of C-reactive proteins, haptoglobin, fibrinogen, plasminogen activator inhibitor and serum amyloid A and sialic acid, as well as cytokines and chemokines.

Exaggerated immune response is responsible for the most of the fatalities in case of coronavirus infection. Release of inflammatory cytokines is contributing to the severity of the disease. The inflammatory cytokine release is primary thought to be due to macrophages[54-56]. Infection by the highly pathogenic respiratory viruses such as SARS-CoV-1, Middle East respiratory syndrome (MERS)-CoV, influenza, and respiratory syncytial virus results in the release of substantial amount of cytokine and chemokine by provoking a prolonged inflammatory macrophage phenotype, allowing for direct viral infection of infiltrating cells. This causes massive cell death and damage of alveolar lung tissue,

increasing morbidity for the patient[57]. Coronaviruses also induce macrophage mediated cytokine storm in patients with type 2 diabetes[58]. Coronavirus infection causes decrease expression of histone methyltransferase, SETDB2 that results in decrease of the repressive trimethylation of histone 3 Lysine 9 (H3K9me3) at NF-κB binding sites on inflammatory gene promoters of inflammatory genes which in turn effectively increases inflammation[58].

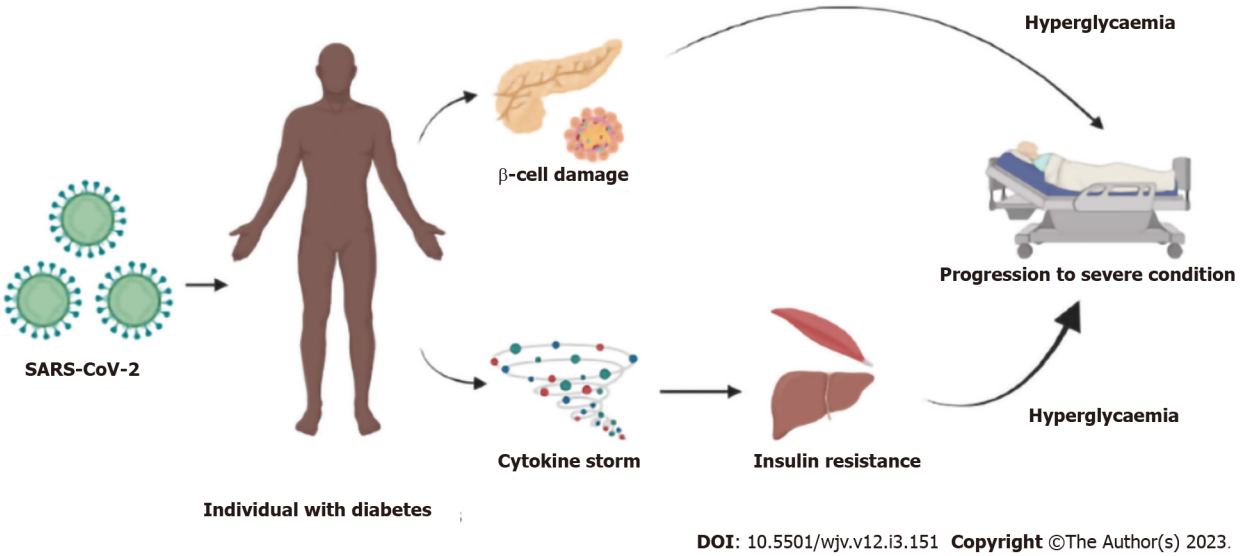
Diabetes is linked to a proinflammatory condition, which may increase the chance of developing severe COVID-19and a higher chance of suffering a cytokine storm. As part of the low-grade chronic inflammation, the proinflammatory cytokines and hazardous metabolites that are present in a cytokine storm are already chronically increased in people with diabetes[59-61]. One of the most significant pathophysiological mechanisms that contribute to higher risk in diabetic patients is believed to involve the proinflammatory NF-kappa-B pathway, which is chronically activated in patients with diabetes[59, 62]. Although the underlying pathogenesis of low-grade inflammation leading to a more rapid progression of COVID-19 and the associated cytokine storm is unclear, it is thought to be one of the most important pathophysiological mechanisms. Interleukin-6, interleukin-8, and tumor necrosis factor- may have the worst prognosis or may be fatal for diabetes individuals among the cytokines that have been studied in research. Increased cytokines are linked to risk factors and comorbidities such hypertension and cardiovascular disease and are associated with greater mortality. In diabetic individuals with COVID-19 who had at least one prior comorbidity, especially hypertension and CVD, management of diabetes by insulin treatment may reduce the rate of mortality among diabetic patients, but it may also be contraindicate[63]. The interplay between SARS-CoV-2 infection and diabetes is shown in Figure 2.

GENETICS OF DIABETES

Diabetes, an endocrine system disease marked by exceptionally high blood glucose levels, is one of the most common diseases in the world. Vascular problems of both the macrovascular system (cardiovascular disease, or CVD) and the microvascular system are the main causes of morbidity and mortality in patients with diabetes (diabetic kidney disease, diabetic retinopathy, and neuropathy)[64]. Despite the complexity and lack of complete knowledge of the precise mechanisms underlying hyperglycemia-induced vascular damage, increased intracellular glucose are thought to result in increased reactive oxygen species production, altering a number of significant downstream pathways, including the flux of the polyol pathway, the formation and activation of advanced glycation end products, the activation of protein kinase C, and the flux of the hexosamine pathway[65].

The HLA has received the majority of attention. in genetic studies of the diabetes, despite the fact that genome-wide association study (GWAS) have so far identified more than 50 Loci that affect Type I Diabetes (T1D) risk[66-69]. PTPN22 possesses a cluster of unusual variants that disrupt mRNA splicing, according to targeted sequencing of known locus, even though no large-scale sequencing projects have been successfully completed in T1D patients[70]. The largest GWAS on T2D to date is a meta-analysis of 32 European cohorts with roughly 74000 cases and nearly 824000 controls. 243 Loci reached genome-wide significance. Together, these top GWAS signals account for more than 17% of the variation in T2D phenotypes, and 73 signals supporting a single causative variable were discovered by thorough fine-mapping research on these loci[71]. People with polygenic scores in the top 2.5% do have a lower probability of getting the condition, despite the fact that these scores, which combine the genetic risk for T2D across numerous genetic loci, are not any more reliable than clinical markers for T2D prediction [72].

The Diabetic Nephropathy Collaborative Research Initiative, led by the GENIE collaboration, published the largest GWAS on diabetic kidney disease (DKD) to date in 2019[73]. The sample size was increased by three times for European Americans with T1D compared to the prior study, and 16 additional significant genome-wide loci connected to multiple illness definitions were found[73]. The missense variant SNP rs55703767 of the type IV collagen alpha 3 chain gene (COL4A3frequent), whose minor allele (T) guards against DKD and numerous other albuminuria-related symptoms, was the one with the highest correlation. Notably, Alport syndrome is caused by loss-of-function mutations in COL4A3[74]. The mutation was also associated with a reduction in glomerular basement membrane (GBM) thickness in a normoalbuminuric cohort for whom ultrastructural data were available for analysis. The SNP rs55703767 had a significantly greater impact in women. In addition, the protective association in an observational study and in those randomly assigned to a conventional *vs* intensive glycemic control in the Diabetes Control and Complications Trial was most pronounced in people with higher hemoglobin A1c (HbA1c) levels, which is noteworthy and might be expected for genetic effects expressed in the context of diabetes[73]. In dissected human glomerulus samples from Pima Indians with DKD and glomerulosclerosis, COL4A3 expression levels were discovered to be negatively correlated with GBM surface density. Furthermore, this study project identified three additional genetic loci that exceeded a strict threshold after controlling multiple correction testing (rs144434404 in intron 1 of BMP7, rs142823282 near TAMM41 and rs145681168 in intron 3 of HAND2-AS1)[73]. The SNP rs144434404 in intron 1 of BMP7, a gene involved in renal morphogenesis that is almost exclusively



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Figure 2 Interplay between severe acute respiratory syndrome coronavirus 2 and Diabetes. Severe acute respiratory syndrome coronavirus 2 infection in diabetic individuals result in β-cell damage and cytokine storm. β-cell damage deteriorates the glucose homeostasis resulting in hyperglycemic condition. The resulting cytokine storm causes insulin resistance particularly in liver and muscles which also results in hyperglycemic condition. These altogether deteriorates the condition of the diabetic individual ensuing increased severity of coronavirus disease 2019. The figure was created using BioRender (https://biorender.com/).

expressed in mouse podocytes, was found to be associated with microalbuminuria. The SNPs rs142823282 and rs145681168, which are located close to TAMM41, as well as rs145681168, which is located in intron 3 of HAND2-AS1, were both substantially linked to microalbuminuria across the entire study. The expression of the neighboring gene PPARG (an expression quantitative trait locus), which is a known T2D GWAS gene but has not been previously studied, was also connected to the TAMM41 signal[74].

With respect to diabetic retinopathy, significant result was found by only one study[75] at the discovery and replication meta-analysis stages by merging two T2D cohorts, one T1D cohort of European ancestry, and one T2D Indian cohort. The genotypes linked to sight-threatening diabetic retinopathy were significantly correlated with genotypes at SNP rs9896052, a variant 17 kb upstream of the *GRB2* gene. This gene encodes an epidermal growth factor receptor-binding protein that is expressed in healthy human retina and is elevated in the retina of a transgenic mice model of retinal stress[76]. Since then, there have been two further comprehensive GWAS of diabetic retinopathy, but at the meta-analysis stage, they have not yet been able to provide definitive proof of a genetic relationship. The genome-wide significant intronic variant SNP rs3913535, which affects NOX4, was discovered by the Genetics of Diabetes Audit and Research in Tayside Scotland (GoDARTS) discovery cohort.

Regarding diabetic neuropathy, the two main GWAS carried out in the GoDARTS project discovered three signals nominally associated with diabetic nerve pain (rs71647933 in ZSCAN20 in female cases and sex-combined analyses only, rs6986153 at chr8q23 in male cases only, and rs17428041)[77,78]. The presence of foot ulcers in people with diabetic neuropathy was compared to two different groups of control people with diabetes and without foot ulcers in a third GWAS using the same GoDARTS dataset. Comparing cases and controls with diabetic neuropathy, the authors discovered the intronic SNP rs80028505 in MAPK14 related with foot ulcers. These studies are from a single cohort, which is significant.

GENETICS OF COVID-19 DISEASE

The COVID-19 is caused by the novel SARS-CoV-2 and was first reported in Wuhan, China. The clinical manifestation of this disease ranges from an asymptomatic to severe disease outcome. Considering the broad spectrum of COVID-19 disease severity, risk factors that predict the disease severity might play a crucial in improving the clinical outcome of COVID-19 patients. Older age, male gender, increased BMI, pre-existing comorbidities and ethnicity are some of the risk factors that are relevant in the context of COVID-19 disease severity and susceptibility. The host genetic predisposition has also been recognized as the crucial risk factor for COVID-19 which is evident by the large number of genome wide association studies, Whole exome sequencing and candidate gene studies conducted by different consortia (COVID-19 Host Genetics Initiative (HGI), Genetics of Mortality In Critical Care (GenOMICC), COVID human genetic effort, independent academic working groups, and commercial genomics service providers such as 23 and Me and Ancestry DNA) regarding the COVID-19 disease to decipher the disease susceptibility

and severity[79].

Human Leukocyte Antigen (HLA) are encoded by the most polymorphic MHC genes. Several HLA alleles have been found to be associated with COVID-19 severity and susceptibility. In a study comprising of 82 Chinese individuals, HLA-C*07:29 and B*15:27 were significantly higher in COVID-19 patients compared to control population after correcting the *P* value[80]. HLA-DRB1*15:01, -DQB1*06:02 and HLA-B*27:07 were significantly higher in 99 severe or critical COVID-19 patients compared to 1017 reference individuals previously analyzed by the research team after applying Bonferroni's multiple test correction[81]. HLA-DRB1*08 was found to be significantly higher in COVID-19 positive individuals and it was also correlated with mortality[82]. HLA-A*11:01, B*51:01, and C*14:02 alleles were found to be significantly associated with worst COVID-19 outcome[83]. In a study comprising of 619 healthy Sardinian controls and 182 SARS-CoV-2 patients, the haplotype HLA-A*02:05, B*58:01, C*07:01, DRB1*03:01 was absent in the patients. The HLA allele HLA-C*04:01 allele and the three-loci haplotype HLA-A*30:02, B*14:02, C*08:02 were significantly more frequently in COVID-19 patients[84].

The cytokine storm has been implicated in severe COVID-19. Seven *IL-6* (rs140764737, rs142164099, rs2069849, rs142759801, rs190436077, rs148171375, rs13306435) variants and five *IL-6R* variants (rs2228144, rs2229237, rs2228145, rs28730735, rs143810642) can be implicated in the pathogenesis and severity of COVID-19[85]. Patients with no previous history of severe infection may develop life-threatening COVID-19 pneumonia due to inborn defects of type I IFN immunity that are TLR3- and IRF7-dependent[86]. None of the asymptomatic patients and 2.1% of severely affected males had the TLR7 deleterious variants[87]. Interferon-alpha and -beta receptor subunit 2 (IFNAR2) variant rs2236757 was associated with critical COVID-19[88].

The ACE2 gene is located at position Xp22.2. The S1 subunit of the spike protein (S) of SARS-CoV-2 binds with the ACE2 on the surface of the host cell. The entry into the host cell requires the cleavage at the S1/S2 site by a cellular serine protease, TMPRSS2[89]. The TMPRSS2 variants rs12329760 was found to have deleterious effect on the protease activity and protective role in COVID-19 patients[90]. The T allele of rs2285666 of ACE2 gene was found to be a risk factor for critical COVID-19 especially for men [91]. The splice site variant rs2285666 was also found to be overrepresented in SARS-CoV-2 positive individuals compared to the 100K genome project controls and in hospitalized European patients compared to outpatients. This variant increases the expression of ACE2. The eQTL rs12006793 was found to be more prevalent in patients in this study[92]. The ACE2 variant (rs190509934:C; a rare X-linked variant) was found to reduce the risk of COVID-19 but not the risk according to a meta-analysis [79].

Ellinghaus *et al*[93] found the 3p21.31 Locus (rs11385942) was associated with severe COVID-19 and respiratory failure in a GWAS study. 3p21.31 spans the gene cluster containing the genes sodium-amino acid transporter 1 (SLC6A20), human leucine zipper transcription factor like 1, CC motif chemokine receptor 9, FYVE and coiled-coil domain-containing protein 1, C-X motif chemokine receptor 6, and X-C motif chemokine receptor 1 genes. A second locus 9q34.2 (rs657152) overlapping with the ABO locus was also found to be significantly associated with severe COVID-19[93]. The GenOMICC (Genetics of Mortality In Critical Care) genome-wide association study involving 2244 critically ill COVID-19 patients from 208 United Kingdom intensive care units, identified significant associations on chromosome 12q24.13 (rs10735079), chromosome 19p13.2 (rs74956615), chromosome (rs2109069) and on chromosome 21q22.1 (rs2236757)[88].

The rs35705950 of the MUC5B was found to be associated with hospitalization in COVID-19. rs35705950 is a promoter specific mutation (https://www.covid19hg.org/results/r6/). The association of rs1886814 in the transcription factor FOXP4 was found to be associated with hospitalization by HGI [94]. Independent of pre-existing dementia, cardiovascular illness, or type 2 diabetes, the ApoE e4e4 homozygous genotype was observed to increase the risk of severe COVID-19[95].

LONG-TERM EFFECTS OF COVID-19 DISEASE

It has been demonstrated that COVID-19's clinical presentation varies greatly, frequently exhibiting substantial respiratory problems. A number of patients with SARS-CoV-2 have gone on to experience long-term problems from the virus, making it notable. Long-haul COVID-19 has evolved to represent a wide range of problems and sequelae of symptoms that may occur, beyond the original reports of individuals feeling weary for months following initial infection[96]. Lung fibrosis, venous thromboembolism (VTE), arterial thromboses, heart thrombosis and inflammation, stroke, "brain fog," dermatological issues, and general mood dysfunctions are some of the potential late consequences that could result from COVID-19 infection, according to prior investigations[97]. Despite the wide range of these long-term problems, certain patient characteristics have been proven to predict which symptoms they would experience and for how long[98].

COVID-19 is mostly a respiratory disease, despite the fact that SARS-CoV-2 can have widespread effects throughout the body. Following COVID-19 infection, numerous long-term pulmonary problems have been reported. Dyspnea, ventilator dependency, oxygen dependence, pulmonary function test (PFT) abnormalities, and fibrotic lung disease are only a few of them. Dyspnea is the most frequent

pulmonary symptom associated with COVID-19, and it might last for two months in 22.9% to 53.3% of patients[99-101]. Infection with SARS-CoV-2 can lead to verifiable long-term alterations in pulmonary physiology in addition to subjective symptoms. Up to 6.6% of survivors who make it to hospital discharge have been found to be oxygen dependent[101]. Long-term weaning from ventilator use is not frequently successful in patients with respiratory insufficiency necessitating a tracheostomy. Only 48% of patients in Spain's 1890 tracheostomy patients were successful in weaning off artificial breathing at the 1-month follow-up[102]. Previous studies have also discussed abnormalities in lung function as determined by PFT. In a study of 55 non-critically ill COVID-19 patients in China, PFT evaluation over a three-month follow-up period indicated abnormalities in 25% of patients, with a decrease in DLCO being the most prevalent (16%)[103]. Salem *et al*[104] made similar observations with elevated incidence of restrictive lung findings when compared with matched controls.

After being released from the hospital following COVID-19, cardiac problems are a frequent complaint. As many as 21% of patients had chest pain 60 days after leaving the hospital, according to Carfì *et al*[99]. Palpitations have also been reported to occur often in as many as 9% of patients at the 60-day follow-up. Aside from the subjective cardiac symptoms listed among the long-term effects of SARS-CoV-2 infection, there have also been a number of quantifiable results. Investigation into the precise prevalence of postural tachycardia syndrome, which has been associated with SARS-CoV-2 infection, is still ongoing[105].

Acute COVID-19 has been linked to a higher risk of thrombotic events, particularly in patients who are severely sick[106,107]. The causes of this coagulopathy are multifaceted and include hypoxia's impact on the activation of hypoxia-inducible transcription factors, microvascular dysfunction, and enhanced expression of tissue factors in response to inflammatory cytokines[108,109]. The regular administration of intermediate-dose anticoagulation (enoxaparin 1 mg/kg) is recommended due to the elevated risk of thrombosis found in this patient population. Recently, a comparison of typical preventive anticoagulation (enoxaparin 40 mg daily) and severely ill individuals with a randomized controlled method in the INSPIRATION trial, COVID-19. Treatment with intermediate-dose anticoagulation did not appear to lower the composite endpoint of arterial or venous thrombosis. ECMO, or death at 30 days as compared to prophylactic anticoagulation at a standard dose[110]. Even though bleeding incidents do occur, most patients benefit more from inpatient VTE prevention than they risk due to the minimal risk of significant bleeding[111,112].

With SARS-CoV-2 infection came a number of long-term neurological and behavioral problems. Two months after the acute infection, patients continued to experience neurological symptoms as fatigue, muscle weakness, difficulty sleeping, myalgia, and headaches, according to long-term symptom data from numerous sources[99,113]. These signs and symptoms have come to represent the long COVID syndrome. In contrast to other viral infections, SARS-CoV-2 infection has also been associated with loss of taste and smell. At a 2-month follow-up, 11% to 13.1% of patients still had chronic loss of taste and smell[100,113]. Due to the considerable burden of severe, life-threatening illness and acute respiratory distress syndrome (ARDS) associated with COVID-19, cognitive disturbances comparable to those shown in ARDS patients in previous studies should be anticipated. At one year of follow-up, ARDS survivors from other causes have reported memory problems (13%), verbal fluency problems (16%), and executive function problems (49%)[114].

A review of the literature found that of the reported cutaneous manifestations of COVID-19 infection, the most prevalent cutaneous manifestation was maculopapular exanthem (morbilliform), which was reported by 36.1% of 72 documented patients in 18 studies. Other cutaneous manifestations included papulovesicular rash (34.7%), urticaria (9.7%), painful red acral purple papules (15.3%), and urticaria, with 19.4% of these manifestations[115]. Another international study of 2560 patients reported that the most prevalent cutaneous manifestation (51.5%) was pernio-like lesions, and that children had a 1.5-day latency period between upper-respiratory infections and cutaneous findings compared to adults, who had a 7.9-day latency period[116]. Only 47 of 1655 hospitalized patients in the Chinese post-acute COVID-19 study (3%) reported skin rashes six months after the infection started[113]; hair loss, on the other hand, was a symptom that was much more frequently reported for patients' months after the COVID-19 infection and was reported in 24 of 120 patients (20.0%) as a post-discharge symptom 110 days after hospital discharge. However, other, more uncommon presentations have been described in case reports, indicating that, although having the same virus in their bodies, the symptoms in various patients may change[117]. Vesicular rashes may be diagnostic of an initial diagnosis of COVID 19 and may be predictive of disease prognosis, although the particular use of these symptoms in this manner has not yet been proven and should be the subject of future prospective research[118].

Children often experience a milder disease during the acute phase of COVID-19 and receive diagnoses at a lesser rate than adults[119]. Multisystem inflammatory syndrome in children (MIS-C), which is characterized by fever and multiorgan dysfunction in the weeks following SARS-CoV-2 infection[120, 121], is one of the consequences and sequelae of infection. 316 cases per 1000000 infections are reported for MIS-C, which primarily affects youngsters from racial/ethnic minority backgrounds[122,123]. Nearly 75% of MIS-C patients require ICU admission, and the condition shares characteristics with both severe acute COVID-19 and Kawasaki illness[120-122]. The most typical symptoms include gastrointestinal issues, cardiovascular issues, respiratory issues, mucocutaneous issues, and neurological issues[122-124].

Poorer COVID-19 outcomes have been linked to pre-existing diabetic mellitus. Meanwhile, COVID-19 has been linked to both type 1 and type 2 diabetes patients' new-onset hyperglycemia and rapid decompensation of diabetes, including diabetic ketoacidosis[125]. In addition to iatrogenic hyperglycemia caused by steroid usage, other suggested mechanisms for hyperglycemia after infection include insulin resistance brought on by the inflammatory state and insulin secretory deficiencies from defective beta cells—caused either directly or indirectly by viral damage[125,126]. Unknown numbers of people with newly discovered diabetes following COVID-19 may have already had undiagnosed diabetes prior to infection, which was merely concealed or made worse by the infection. It's also not certain whether diabetes that develops after being hospitalized for COVID-19 is a lifelong condition. In order to better understand the relationship between COVID-19 and diabetes and to better define the length of post-COVID-19 diabetes, the global CoviDiab Registry was established[127].

Acute COVID-19 patients frequently experience acute kidney injury (AKI), and 5% of all hospitalized patients need inpatient renal replacement treatment[128]. The causes of AKI are multifactorial, and they include aberrant coagulation, systemic hypoxia, inflammatory cytokine effects, and direct virus harm [129]. The most frequent histological finding is acute tubular necrosis, however glomerulopathy[130] and microvascular thrombi[131] can also occur[132-136]. AKI is linked to an increase in hospital mortality, and those who make it out of the hospital[128].

SHARED VARIANTS RESPONSIBLE FOR DIABETES AND SEVERITY OF COVID-19 DISEASE

Frequencies of variations in human ACE2 receptor gene is not uniform rather varied considerably from population to populations. Mutations emerging within the SARS-CoV-2 genome and natural polymorphisms harbored within the ACE2 receptor gene are crucial for the binding of receptor binding domain of spike protein of SARS-CoV-2 followed by the entry and transmission of the virus[137]. Several studies have been conducted to evaluate the binding affinity of the novel SARS-CoV-2 variants to hACE2[138-140] and association of population specific ACE2 variants with disease susceptibility as well as severity has been reported[141-143]. Presence of ACE2 receptor polymorphisms can alter binding interaction with SARS-CoV-2 followed by COVID-19 disease susceptibility[144] on one hand while on the other side different mutations in SARS-CoV-2 revealed varied binding pattern to its host receptor, ACE2[145].

Studies on different population revealed linkage between the single nucleotide polymorphisms within the angiotensin converting enzyme 2 receptor gene and hypertension, diabetes, cardiovascular diseases[146,147]. The ACE2 variants rs2285666, rs879922, rs4646188, rs2106809, rs4240157, rs4830542, rs2158083, rs879922, rs1514283, rs2074192, rs4646155, rs4646176, rs4646174 and rs233575 have been reported to be associated with primary hypertension while rs2106809, rs2074192, rs4646156, rs879922, rs4240157 and rs233575 were found to be linked with left ventricular hypertrophy[148]. Incompatible results have been reported regarding association of *ACE2* gene variants with the risk as well as severity of COVID-19 disease. Studies conducted on Turkish, Italian and Spanish populations reported that *ACE2 receptor* gene rs2106809, and rs2285666 polymorphisms were not associated with the severity of COVID-19 infection[148-151] while ACE2 rs2285666 (AA allele) and ACE2 rs2074192 (TT allele), and for ACE2 rs4646174 (GG allele), ACE2 rs4646156 (TT allele) and ACE2 rs2158083 (TT allele) the most significant correlation with COVID-19 in Polish population[152]. On the other hand, in another study Cafiero *et al*[153] reported SNPs within the members of renin angiotensin system such as rs2074192 within ACE2, rs1799752 within ACE1 and rs699 within angiotensinogen, SNPs could potentially be a valuable tool for predicting the clinical outcome of SARS-CoV-2 infected patients.

Intronic variant rs228666 (located in intron 3) can cause change in mRNA splicing followed by altered ACE2 expression which ultimately results in higher binding affinity to novel coronavirus[152] and similar characteristics in case of rs2158083 was observed in patients with arterial hypertension[138]. Another intronic variant rs2074192 has been demonstrated to dysregulate SARS-CoV-2 binding to its host receptor by mediating imbalance in ACE2 transcription/translation through inducing changes in secondary structure of RNA[138,152]. Sienko *et al*[152] demonstrated significant correlation of the *ACE2 receptor* gene rs2074192, rs2158083, rs2285666, rs4646156, rs4646174 polymorphisms with the severity of COVID-19 in adult patients. Strong correlation of T allele and TT genotype with respect to of rs2074192 within *ACE2 receptor* gene has been shown with disease severity caused by SARS-CoV-2[151,152] and in French-Canadian and British patients (n = 1644) with COVID-19 disease in obese smoking males[154]. Recent research has demonstrated that persons with type 2 diabetes (T2D) experience COVID-19 with greater severity and mortality as compared to healthy individuals. Patients with T2D who are infected with SARS-CoV-2 are more prone to experience severe cytokine storm consequences and need to be hospitalized to high-dependency or intensive care facilities. Due to the severe activation of inflammatory cascades, some COVID-19 individuals are known to experience different types of acute respiratory distress syndrome and have a greater mortality risk.

Recently, Wu *et al*[155] tried to find the common genetic determinants between T2D and COVID-19. They analyzed the key pathways that were shared between T2D and COVID-19 in order to identify the common pathways between the two diseases. Using iGSEA4GWAS, they discovered chemokine binding, G-protein coupled chemoattractant receptor activity pathways (CCR2 and CCR3), TFAP2 family pathway (TFAP2B), and ventricular cardiac muscle cells differentiation (RARB and PROX1) pathways shared by T2D and COVID-19. Immunological cell recruitment to infection sites is aided by the chemokine binding pathway, and leukocyte chemotaxis and innate and adaptive host immune responses are mediated by the G-protein coupled chemoattractant receptor. Additionally, using PASCAL, 15 pathways that were common between T2D and COVID-19 were discovered. These pathways were linked to a number of biological functions and organs, including the heart, axons, and calcium channels. However, no shared pathways between the four pathway-based analytic programs were discovered. 394 genes were associated with T2D according to PASCAL analysis, while 58 genes were associated with COVID-19. Five were discovered to be common between T2D and COVID-19 after comparing the important genes in T2D and COVID-19: PTPRD, CSMD1, MAGI1, ASIC2, and DAB1.

Significantly positive genetic correlations between T2D and COVID-19 were found in genome-wide linkage disequilibrium score regression studies (R for genetic = 0.15, *P* value = 0.01). There is a slight polygenic overlap between COVID-19 and T2D, as evidenced by the enrichment of associations with COVID-19 across different degrees of association with T2D[22]. Wu *et al*[155] further revealed two loci shared between COVID-19 and T2D: ABO (rs505922, intronic) and NUS1 (rs3924604, intronic) based on a threshold of conjFDR<0.05. Both of these independent loci were displaying a consistent direction when the effect directions of the shared independent loci were compared (conjFDR<0.05). One locus (rs505922) has shown a favorable effect, whilst the other (rs3924604) demonstrated a detrimental effect. SNPnexus was used to identify these two genes from two separate SNPs. In the genes closest to the identified loci shared between COVID-19 and T2D, eight pathways were found to be significantly overrepresented, with the faulty DHDDs causing retinitis pigmentosa 59 pathway being the most significant (*P* value = 1.88×10^{-4}).

CONCLUSION

The world has overcome the devastating impact of COVID-19 by undergoing massive vaccination program against SARS-CoV-2. The pandemic has also showed us weaknesses of the health care system of both the developed and underdeveloped world. Also, we should think about preserving our forests, water reservoirs *i.e.*, the natural habitats of all living beings. To do so, we need to revisit the actions of climate change. Moreover, the world researchers, policy makers, health care providers should also consider the probable impacts of long COVID on human health. Understanding the genetics of hosts and disease pathophysiology could be one of the many steps towards better human health and well-being.

FOOTNOTES

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REVIEW

Viruses and autism: A Bi-mutual cause and effect

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Abstract

Autism spectrum disorder (ASD) is a group of heterogeneous, multi-factorial, neurodevelopmental disorders resulting from genetic and environmental factors interplay. Infection is a significant trigger of autism, especially during the critical developmental period. There is a strong interplay between the viral infection as a trigger and a result of ASD. We aim to highlight the mutual relationship between autism and viruses. We performed a thorough literature review and included 158 research in this review. Most of the literature agreed on the possible effects of the

viral infection during the critical period of development on the risk of developing autism, especially for specific viral infections such as Rubella, Cytomegalovirus, Herpes Simplex virus, Varicella Zoster Virus, Influenza virus, Zika virus, and severe acute respiratory syndrome coronavirus 2. Viral infection directly infects the brain, triggers immune activation, induces epigenetic changes, and raises the risks of having a child with autism. At the same time, there is some evidence of increased risk of infection, including viral infections in children with autism, due to lots of factors. There is an increased risk of developing autism with a specific viral infection during the early developmental period and an increased risk of viral infections in children with autism. In addition, children with autism are at increased risk of infection, including viruses. Every effort should be made to prevent maternal and early-life infections and reduce the risk of autism. Immune modulation of children with autism should be considered to reduce the risk of infection.

Key Words: Autism; Children; Rubella; Cytomegalovirus; Herpes simplex virus; Influenza virus; Zika virus; SARS-CoV-2; COVID-19; Viral infection

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Core Tip: There is a mutual relationship between viral infections and autism. There is an increased risk of developing autism when contracting a viral infection during pregnancy or early postnatal life during the critical period of brain development. At the same time, children with autism have many co-morbidities that expose them to more risk of contracting infections, including viruses. Therefore, every effort should be made to prevent infections, especially during this critical period of neurodevelopment. Parents should also be educated about the importance of vaccination and immune modulation in children with autism to avoid further infections.

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INTRODUCTION

Autism is a group of heterogeneous, multi-factorial, neurodevelopmental disorders that occur during infancy and toddlerhood, best described as a spectrum rather than a disease. It is characterized by language, social communication, interaction problems with restricted, repetitive, or stereotyped behaviours or interests, and the inability to generate biologically determined, regular, emotional interaction with others[1]. Autism spectrum disorders (ASD) involve autism, pervasive developmental disorder not otherwise specified, and Asperger's disorder. In addition, ASD, childhood disintegrative disorder, Rett's disorder, and the overactive disorder accompanied by mental retardation and stereotyped movements form pervasive developmental disorders. The diagnosis of autism is made by identifying at least two domains (out of three) of impaired social interaction and/or communication and restricted, repetitive, or stereotyped behaviour, interests, and activities[2,3].

There is no one definite cause of autism. Several factors play together to increase the risk of development of autism, including genetic, epigenetic, and environmental factors that may work in combination to affect the brain during the crucial phases of early development. The genetic causes of autism could result from single-gene mutations or abnormal copy number variations (such as large deletions, duplications, inversions, or translocations of chromosomes)[4]. As autism is likely to run in families, specific genetic changes may increase the risk of autism development in children. Therefore, the risk of recurrence of pervasive developmental disorders ranges between 2% and 8% in siblings of children with autism[5]. Lichtenstein *et al*[6] showed that monozygotic twins had higher rates of ASD and other neuropsychiatric disorders than dizygotic twins. However, this increase in the risk is not only due to the shared genes but also could be related to the shared environment, as identified by several twin studies[6,7].

Therefore, specific environmental conditions may increase or decrease the risk of autism in genetically predisposed patients through epigenetic modification by affecting gene expression quantity and quality without altering the DNA sequence. The epigenetic modification of gene expression occurs through altering DNA methylation, changing histone proteins, or modifying the expression of noncoding RNAs[8]. According to Barbeau[9], three pathways trigger the development of autism. The

first pathway is activated by in-utero insult or injury, while obstetric complications at birth initiate the second pathway. The third pathway is triggered by various environmental triggers affecting infants in the first three years of life. The environmental factors include antennal factors (*e.g.*, parental age, especially the father's age, the mother's physical and mental health, prenatal drug use, and family socioeconomic status), prenatal factors (preterm delivery, abnormal presentation, cesarean section, fetal complications, neonatal hypoxia, respiratory distress, natal bleeding, low-birth weight, seizures at birth), and postnatal factors (*e.g.*, neonatal jaundice, early infection, sepsis, meningitis, encephalopathy, postnatal vitamin D deficiency, *etc.*)[10].

The sharp rise in ASD incidence observed worldwide is due to the increased prevalence of risk factors such as genetic predisposition, adverse environmental circumstances, and increased awareness about this disorder[11]. Infection is a common triggering factor in the three environmental pathways that affect autism development. Viral infection is widespread in all ages, including during the antenatal period. Viral infection during critical periods of early in-utero neurodevelopment may lead to an increased risk of autism in the offspring. On the other side, autism may be associated with impaired cellular and humoral immunity, which predisposes children with autism to encounter different types of infections, including viruses[12].

Meanwhile, some viruses may be used as a vector during gene therapy which could be a promising therapy for many diseases, including autism[13]. This review highlighted the mutual relationship between autism and viruses. The study focused on how viral infections may affect brain development and how this could be at play in some traits commonly associated with autism, such as difficulty communicating verbally or recognizing familiar faces. It also focused on how children with autism may have an increased frequency of infections, especially viral infections.

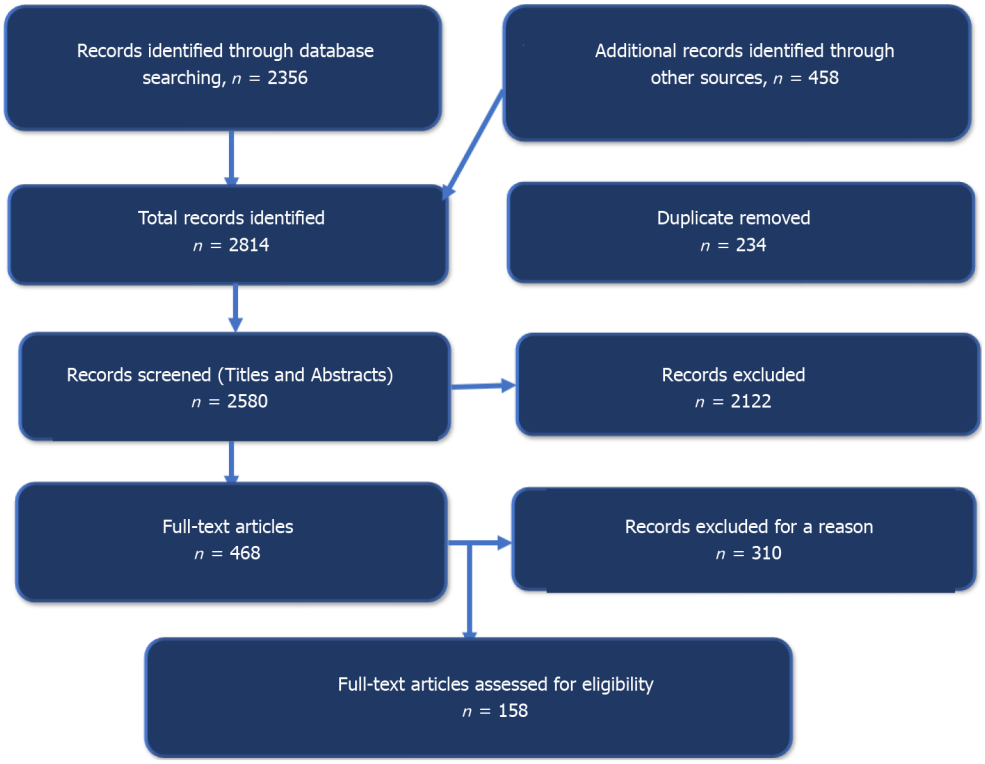
LITERATURE REVIEW

We conducted an inclusive literature review by searching different electronic databases, including Embase, PubMed, Cumulative Index to Nursing and Allied Health Literature, Cochrane Library, Scopus, Web of Science, Library and Information Science Abstracts, the National Library of Medicine catalogue, Ovid/Medline, and google search until January 31, 2023, related to viral infections and autism using the terms autism, autism spectrum disorders, viral infections, rubella, cytomegalovirus, influenza virus, Zika virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), coronavirus disease 2019 (COVID-19), vibrobiota, neurodevelopment, perinatal, antenatal, natal, and postnatal. Reference lists were inspected, and citation searches were done on the included studies. We included papers written in English and with open access. Figure 1 shows the flow chart of the reviewed articles. We reviewed 458 articles concerned with the viral infection and autism in children; 158 were included in the study.

PROPOSED UNDERLYING PATHOPHYSIOLOGY OF AUTISM

ASD pathogenesis is not fully recognized. Autism has different pathophysiological neuroanatomical, and neuropsychological changes in the affected patients' brains that affect many brain functions resulting in the characteristic cognitive and behavioural changes of autism. Different genetic and environmental factors activate pathological pathways that disrupt brain development[14,15]. Behaviour and social impairment are among the hallmarks of autism. ASD is diagnosed based on behavioural impairments in social communication, interest fixation, and repetitive behaviours. These social impairments may be related to the improper interpretation of social signals[16]. Evidence from healthy individuals suggests that potentially threatening situations, such as others' proximity, can trigger several physiological responses that help regulate the distance between themselves and others during social interaction. Vicarious fear learning critically impacts cognitive abilities, receiving a neutral image as threatening and frightening as phylogenetically innate negative and dangerous stimuli, consequently affecting the person's behavioural control[17,18]. Individuals with ASD have social impairments, potentially due to the lack of or improper social signal interpretation, resulting in the inability to interpret these signals to guide appropriate behaviours[19]. In utero or early life, disruption of normal brain development triggers the subsequent development of neuropsychiatric disorders during later life [20].

The brain volume and weight are usually more prominent in children with autism with larger head circumferences than in typically developed children. The brain overgrowth observed in children with autism is not precisely understood. However, it could be related to excess neurons that induce local overconnectivity in specific brain regions. It also could be related to abnormal neuronal migration during early pregnancy with abnormal synaptogenesis and formation of dendritic spines and disturbed excitatory-inhibitory networks[21-23]. Changes in the division rates of germinal cells induce abnormal daughter cells migration to their target regions, causing autism-associated sensory and motor deficits [24].



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Figure 1 Shows the flow chart of the reviewed articles.

Neuroinflammation is common in ASD with low-grade chronic inflammatory reactions and increased pro-inflammatory cytokines in cerebrospinal fluids (CSF) and specific brain areas due to microglial cells and innate neuroimmune system activation[25]. Altered immune function induces neuroinflammation, affecting many neurological processes, including neural development, brain structures, synapse plasticity, cognition, and behaviour[26]. This neuroinflammation may begin in early embryogenesis during the critical periods of neurodevelopment, resulting in unsuccessful neurodevelopment. Various antenatal factors, such as maternal vitamin D deficiency, medication use, such as valproic acids, prenatal infection, and neonatal hypoxia, can trigger autism-related neuroinflammation. Brain development during the perinatal period is critical and particularly vulnerable to the effects of abnormal immune activation with detrimental consequences on neurodevelopment and alterations in neural connectivity[27]. Gastrointestinal abnormalities, repeated infections with gut dysbiosis, and impairment of the gut-brain axis cause immune imbalance and trigger neuroinflammation[28]. The role of neuroinflammation in autism pathogenesis is proved by observing the raised reactive microglial and astrocyte numbers in postmortem tissue from patients with ASD and animal models[29]. Other evidence of neuroinflammation is frequent reporting of dysregulated immune responses, anti-brain antibodies in CSF and blood, and several neurotransmitter abnormalities, such as increased serotonin levels in children with autism. Neuroinflammation identification and other markers of immune profile abnormalities can hypothetically lead to more consistent diagnostic measures and options for treating ASD[30,31].

The mirror neuron system (MNS) is essential in developing social and communication skills by helping to understand other people *via* imitating their behaviour through personified simulation, intentions, action perceptions, and emotions. Children with autism were found to have structural abnormalities in MNS regions, causing impaired activation of the imitation core circuit in children with autism. Remarkably, the activity of MNS regions is inversely related to the severity of the deterioration of social domain communication, which can explain the social impairment in children with autism[32,33]. However, children with autism can still mimic goal-directed behaviours[34]. In addition to the impaired MNS observed in children with autism, they have impaired activation of many other brain circuits outside the MNS[35]. For example, patients with autism may have an altered functional organization of their large-scale task-negative brain network engaged in social and emotional processing. However, they have an intact organization of the task-positive brain network, which maintains attention and goal-directed thinking[36]. Therefore, it was unsurprising when Chiu *et al*[37] found different signalling patterns in the cingulate cortex using functional neuroimaging with severely reduced cingulate self-response when playing games in patients with autism from that observed in typically developed partners. Some studies showed that patients with autism might have local overconnectivity in the cortex with reduced functional connectivity between the frontal lobe cortex and the rest

of the cerebral cortices with poor high-level neural connectivity and synchronization and disordered association cortex[38,39].

Neuroinflammation of the brainstem causes brainstem dysfunction, including sensory processing abnormalities[40]. Since the thalamus is close to the brainstem, neuroinflammation could also affect it, augmenting autonomic nervous system dysfunction. Therefore, children with autism have intermittent autonomic nervous system dysfunction with enhanced sympathetic excitation and parasympathetic hypofunction causing chronic sensory hyperarousal state in children and sleep disorders. Autonomic dysfunction also affects heart rate, blood pressure, respiratory rhythm, gastrointestinal motility, gastric acid, and intestinal enzyme secretion[41,42]. Immune dysregulation and autoimmunity can induce neuroinflammation, cytokine dysregulation, and inducing anti-brain antibodies, significantly impacting brain development, causing neurodevelopmental deficits and playing a role in autism progression[43]. Vargas *et al*[44] showed the presence of neuroinflammatory markers and elevated cytokines, and enhanced activity of microglia and astrocytes in postmortem autopsies from patients with autism between 5 to 44 years, indicating permanently activated immune status in patients with autism. Table 1 summarizes the possible underlying mechanisms and triggering factors for autism.

VIRAL INFECTION AS A TRIGGER OF AUTISM

Increased susceptibility to infection is a common problem during pregnancy due to various pathophysiological and mechanical changes and immune system adaptation necessary to keep the fetus in utero and prevent expulsion. Therefore, there is an increased chance of asymptomatic and symptomatic viral infection during pregnancy[45]. Some viruses can cross the placental barrier, reaching the fetus and causing devastating developmental fetal effects[46]. Infection is an important trigger for immune activation. Viruses can directly infect the brain, causing neuron cell death by cell lysis, the release of free radicals, or apoptosis induction, inducing a systemic inflammatory response that affects the brain or alters the maternal or their offspring's immune status, which could influence autism development. Viral-induced immune activation causes elevated levels of pro-inflammatory cytokine IL-6, which changes brain gene expression in the offspring, driving abnormal behaviour development[47]. Maternal immune activation increases maternal pro-inflammatory cytokines, activates maternal T-helper-17 cells, and increases IL-6 mRNA and protein in the fetal brain and the placenta making specific placental tissue changes commonly observed in patients who developed ASD[48-51]. Some viruses, such as rubella and cytomegalovirus, may have a teratogenic effect on the fetus, impairing brain functions and causing autism[46]. The brain and immune system are not fully developed in fetuses and young infants, so they are at high risk of viral-induced brain damage. Therefore, maternal viral infection and inflammation during critical periods of pregnancy could result in an unfavourable intrauterine environment and alter the brain structure and function, raising the risks of having a child with autism[52]. The effects of maternal infections depend on many factors, including genetic susceptibility, the time of infection in pregnancy (early or late), and the severity of the infection [53]. Figure 2: General mechanism of viral infection in the induction of autism.

More than 50 years had elapsed since the link between viral infection and autism development appeared on the surface when Chess *et al*[54] observed the development of autism in 7% of children suffering from congenital rubella. One year later, Deykin and MacMahon[55] hypothesized that direct exposure to or getting infected with prenatal rubella, measles, mumps, or postnatal mumps might have a causal role in developing autism. After that, many studies documented the link between other viral infections, such as polyomaviruses, cytomegalovirus, and influenza, with the increased risk of autism [56,57]. Many animal studies have shown the role of prenatal or early postnatal exposure to infections, including viruses, in developing permanent neurological and behavioural abnormalities in offspring involving autism features[57,58]. Shi *et al*[57] showed that pregnant mice infected with the human influenza virus give up offspring with significant behavioural abnormalities as adults, including schizophrenia and autism, with deficits in prepulse inhibition in the acoustic startle response. Moreno *et al*[59] showed that antenatal mice-adapted influenza virus infection could induce behavioural changes by altering serotonin and glutamate systems *via* upregulating serotonin 2 A receptors and downregulating metabotropic glutamate receptor 2 in the frontal cortex of the born mice.

There is increasing evidence that a prenatal infection can lead to autism. Maternal infection by DNA or RNA viruses that can cross the maternal-fetal interface may induce neurodevelopmental problems in the developing fetus[60]. A study published in the American Journal of Perinatology 2017 Looked at data from more than 100000 children who were a part of the Kaiser Permanente health system in California from 1991 to 2009. It showed that women who developed an infection in the third trimester were at an increased risk for developing autism in their children[61]. Another study by Croen *et al*[62] showed that the risk of autism is double in mothers with second-trimester infections and fever, suggesting that the infection-induced inflammation during a specific temporal window of pregnancy may be an etiological factor. Fang *et al*[63] studied 4184 children with ASD, and 16734 healthy, controlled-matched children over seven years between 2000 and 2007 to evaluate the link between prenatal infections and the risk of developing autism across three trimesters. They showed that

Table 1 The possible underlying mechanisms and triggering factors for autism

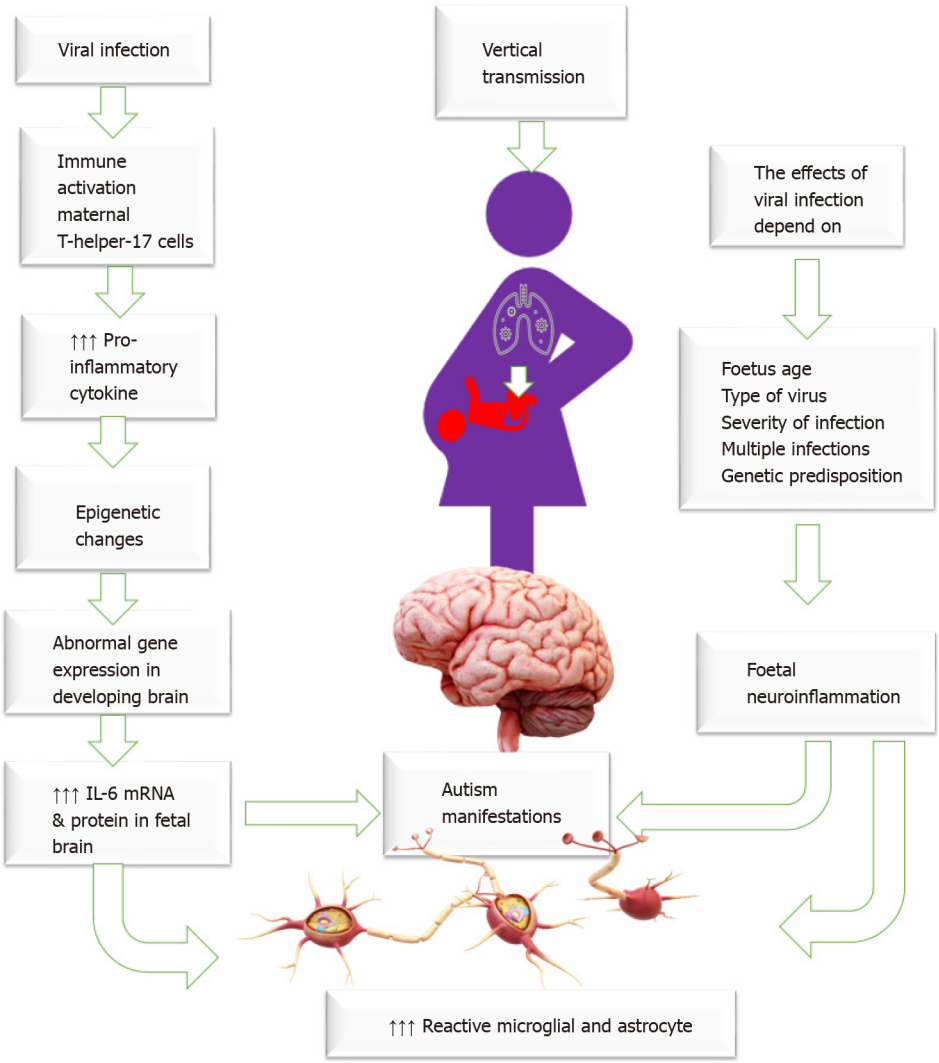
Possible mechanism	Effects
Immune dysregulation and autoimmunity	Neuroinflammation, cytokine dysregulation, and inducing anti-brain antibodies
Neuroinflammation	**Neuroinflammation of the Cortex:** Raised reactive microglial and astrocyte numbers → excess neurons → local overconnectivity in specific brain regions, abnormal neuronal migration during early pregnancy → abnormal synaptogenesis and formation of dendritic spines and disturbed excitatory-inhibitory networks, prominent brain volume and weight volume
	Neuroinflammation of brainstem → brainstem dysfunction → sensory processing abnormalities → enhanced sympathetic excitation and parasympathetic hypofunction
	Neuroinflammation of thalamus → autonomic nervous system dysfunction
Abnormalities in mirror neuron system regions	→ Impaired activation of the imitation core circuit → social impairment
Impaired signaling patterns in the cingulate cortex	→ Severely reduced cingulate self-response → social impairments
Autonomic nervous system dysfunction	Chronic sensory hyperarousal state in children and sleep disorders
	Affects heart rate, blood pressure, and respiratory rhythm
	Impaired gastrointestinal motility, gastric acid, and intestinal enzyme secretion
Underlying triggering factors	Maternal vitamin D deficiency, use of medication such as valproic acids during pregnancy, prenatal infection, neonatal hypoxia, preterm delivery, abnormal presentation, cesarean section, fetal complications, neonatal hypoxia, respiratory distress, natal bleeding, low-birth weight, seizures at birth, neonatal jaundice, early postnatal infection, sepsis, meningitis, encephalopathy, postnatal vitamin D deficiency
Augmenting factors	Gastrointestinal abnormalities, repeated infections with gut dysbiosis, and impairment of the gut-brain axis cause immune imbalance and trigger neuroinflammation

contracting infections in the third trimester was slightly associated with a raised risk of ASDs. However, the effects of infection in the third trimester on the risk of autism development demands further exploration. Zerbo *et al*[64] showed that maternal infections during pregnancy, especially bacterial and multiple infections that need hospitalization, are associated with an increased risk of having a child with ASD. There is some evidence that boys are at a more increased risk of antenatal viral infection and, consequently, autism, primarily due to the noted sex bias seen in the placental immune response related to the viral infection[65]. The placenta in female babies can induce random X- chromosome reactivation, potentially increasing the gene dosage of specific X-linked genes at a very early or late pregnancy period, inducing enhanced protection against viral infection[66].

It should be noted that most of the included studies give evidence from an epidemiological perspective. It is essential to consider possible confounding factors and other disorders during pregnancy, such as preterm birth, low birth weight, maternal smoking, and seasonal variation. An interesting study showed that the risk of autism was highest in babies conceived in the winter and born in the fall, while the rate was lowest in babies conceived in the summer and born in the spring[67]. Could the rate be related to an increased incidence of infection during winter time or low exposure to sunlight during a period of critical neurodevelopment? This finding needs further exploration.

Not only does infection during pregnancy increases the risk of developing ASD, but infection in early postnatal life is also associated with increased risk. A study by Getahun *et al*[68] showed that preterm and term babies who encountered neonatal sepsis had a significantly increased probability of autism for both boys and girls and in all race-ethnic groups except Asian/Pacific Islanders compared with unexposed children. A case-control Danish study involving 414 children with ASD cases and 820 children as controls showed that children with autism had a higher risk of infection-related hospital admission during the first year of life than the controls[69]. However, another study by Rosen *et al*[70] showed an increased risk of autism with infection during the first 30 days postnatal and not the first two years of life.

On the other hand, some previous studies showed no relation between antenatal viral infection and the risk of autism. Anlar *et al*[71] found no association between antibody levels against Human parvovirus B19 and the risk of autism in 22 children with autism and 50 children with other neurological disorders as control. However, they did not compare children with autism with typically developed children, which is a substantial limitation of the study. In addition, Sylvester Jørgensen *et al*[72] found no significant differences in the prevalence of herpes simplex virus antibodies in 123 children with different psychiatric disorders, including autism, and 86 typically developed children. Meanwhile, Deykin and MacMahon[55] suggested that antenatal or early postnatal exposure to chickenpox, mumps, measles, or rubella did not increase the likelihood of developing autism. However, they proposed that combined infection of two or more of these viruses could increase the risk of autism, especially for antenatal mumps, measles, rubella, and postnatal mumps. However, there may be a limitation in these



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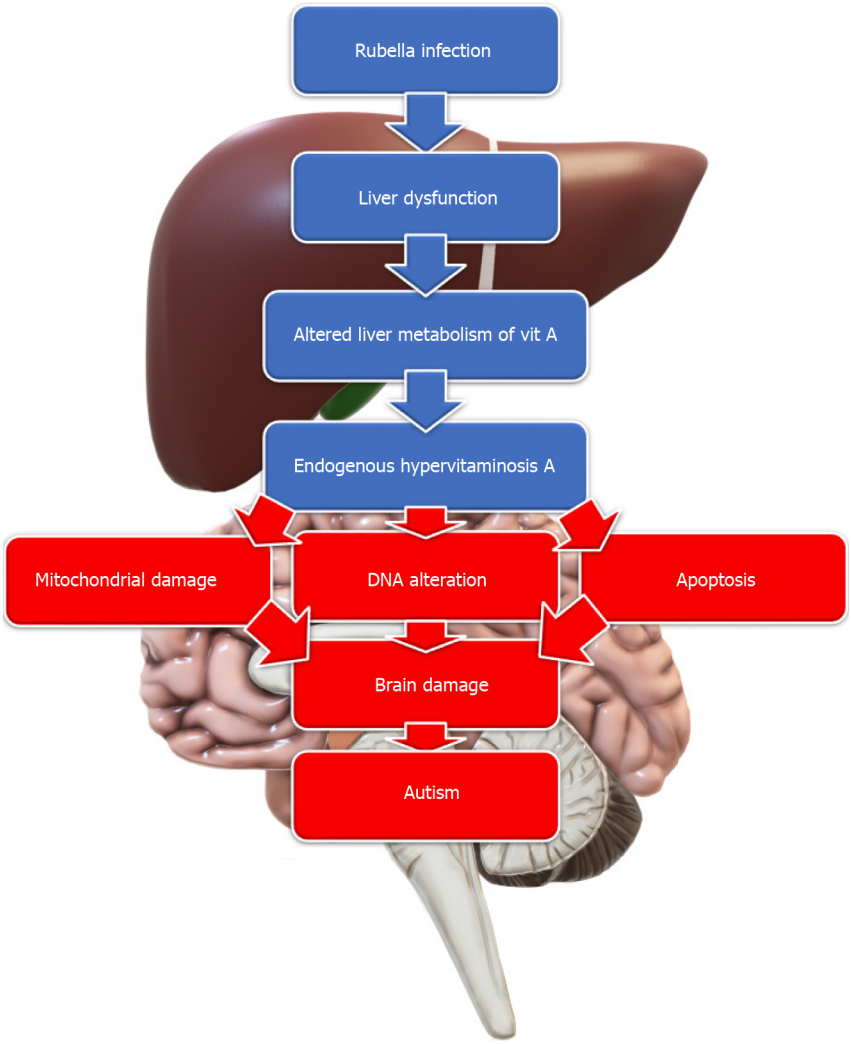
Figure 2 General mechanism of viral infection in the induction of autism. The figure showed that maternal viral infection could reach the virus through vertical transmission and affect the placenta. It also causes maternal immune reactivation with activation of the maternal T-helper -17. This immune hyperactivation leads to a marked increase in the pro-inflammatory cytokines causing epigenetic changes in the fetus, which cause abnormal gene expression in the developing brain with overactivation of astrocytes and glial cells and the development of autistic manifestations.

studies' methodology that could explain the contradictory results with recent studies.

Specific viral infections and the risk of developing autism

Certain viruses are known to be more commonly associated with the development of autism.

Rubella: Rubella is a well-known RNA virus that can cause various congenital malformations when contracted, especially during the first trimester of pregnancy. Intellectual disabilities, including autism, are common among children with congenital rubella infection. The autism rate is 200 times more in these children than in the general population, reaching a rate of 8%-13% of children with congenital rubella syndrome[73]. Different mechanisms were proposed, including infection-induced hypervitaminosis A. Acute rubella infection induces mild liver dysfunction altering the liver metabolism of vitamin A with the spilling of the stored vitamin A complexes into the circulation, resulting in an endogenous type of hypervitaminosis A, which serves as a teratogen causing mitochondrial damage, DNA alteration, and apoptosis; inducing autism development[74] (Figure 3). In addition, the rubella virus can invade and replicate inside the brain cells. It also can cause cerebral vascular lesions and haemorrhages. It can also cause fulminant degeneration of leptomeninges with significant brain volume loss and destruction of white matter[73]. Therefore, many manifestations of congenital rubella syndrome, such as congenital heart defects, spasticity, deafness, and visual impairment, are common in children with autism. In addition, radiological findings, such as dilated perivascular spaces, periventricular leukomalacia, calcifications, and decreased perfusion to specific brain areas, are common for children with congenital rubella syndrome and those with autism[73]. Hence, rubella might still be a possible cause of autism, even in countries with well-vaccination policies.



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Figure 3 The effect of antenatal Rubella Infection on the brain. Rubella virus causes liver dysfunction, which induces altered vitamin A metabolism and endogenous hypervitaminosis A. This hypervitaminosis A causes mitochondrial damage, DNA alteration, and altered apoptosis, resulting in brain damage and autism manifestations.

Due to the high incidence of autism in children with congenital rubella infection, they should be followed strictly for signs of autism. Symptoms of autism may not appear till adolescence. Conversely, clinicians should screen children with autism for possible signs of congenital rubella infection[75]. This delay in developing congenital rubella infection manifestations could be related to persistent rubella virus infection in the affected organs or developing autoimmune responses to an old infection[76]. Even though the United States of America was declared free from congenital rubella syndrome in 2004, the rate of autism is increasing in the USA, reaching a rate of 1:59[77]. The increased autism rate may indicate that subclinical rubella still plays a role, or at least rubella infection is just one of the players.

Cytomegalovirus: Human cytomegalovirus (CMV) is a double-stranded DNA neurotropic beta-herpes virus. It is the most prevalent member of the human herpesvirus family with high species-specificity as a human is the only host. Therefore, it is recently known as human herpes virus 5 (HHV-5)[78]. Congenital CMV infection is one of the leading infectious causes of neurological impairment in the new coming baby, with an infection rate between 0.5% and 1%. Congenital CMV infection can produce various clinical manifestations at birth in 10%–15% of the infected babies. However, asymptomatic infections can be reactivated anytime throughout life[79]. Engman *et al*[80] showed that congenital CMV infection is present in 3% of children with autism. CMV infection blocks interferon production and impairs CD8 T-cell function, thus inhibiting the antiviral defense mechanism of the host and inducing inflammation both in the maternal circulation and the placenta. In addition, it causes local infiltration of macrophage and T-cells at the site of infection *in vivo*, altering immune function and playing a significant role in perinatal brain injury[81,82]. CMV infection-associated alteration of the immune response causes disrupted development of particular regions or structures in the brain that lead to the development of autism[83].

CMV infection increases the circulating maternal pro-inflammatory cytokine levels, which is associated with an increased risk of autism and other mental disorders[84]. Children with congenital CMV infection may have many typical features of autism, including poor development of adequate interpersonal relationships, weak eye-to-eye contact, impaired language development, and non-thematic use of objects[85]. The presence of high CMV-IgM levels may indicate maternal infection with CMV[86]. Neonates with suspected congenital CMV could be diagnosed by detecting CMV-DNA in the cord blood or their urine in the first postnatal two weeks using real-time polymerase chain reaction[87,88]. Children with autism suspected of having congenital CMV infection may also have positive CMV-DNA in the urine and positive CMV- IgM antibodies in their serum[89,90]. Cranial ultrasound may show calcifications, increased periventricular echogenicity, ventriculomegaly, and intraventricular adhesions[91]. Magnetic resonance imaging (MRI) of their brain may reveal the presence of abnormal periventricular white matter intense areas, suggestive of disturbed myelination. MRI brain may also show an increased rate of hippocampal abnormality in children with congenital CMV infection. MRI may be normal in the first few weeks of life in children with congenital CMV; however, It may show the characteristic changes in white matter from temporal to posterior regions by one year of age[92,93]. Therefore, at birth, asymptomatic children might develop autism within the first few years of life[94].

In addition to congenital infection, CMV infection in infancy may raise the risk of developing autism. Postnatal CMV infection can impair overall cognitive functions and reduce intelligence during childhood and adolescence[58]. Lin *et al*[95] showed that CMV infection in infancy might raise the risk of consequent autism and epilepsy but not attention deficit hyperactivity disorder, particularly in infants under two years old. Yang *et al*[96] also showed that postnatal CMV infection might raise the predisposition to tuberous sclerosis and autism spectrum disorders. Both epilepsy and tuberous sclerosis are associated with an increased risk of autism[97].

Herpes simplex virus: The herpes simplex virus (HSV) is divided into two main types; HSV-1 and HSV-2. HSV-1 is mainly transferred through the oral route, while HSV-2 is principally transmitted through sex. About 67% of the global population has HSV-1 infection, while 13% have HSV-2 infection worldwide. About 15% of women of childbearing age are seropositive against HSV-2 globally[98]. HSV induces the release of inflammatory molecules and immune system activation that alter the brain structure with abnormal growth of the cerebral neocortex, a common finding in children with autism, especially during the first 6-12 mo of life[99]. A Norway study between 1999 and 2008 showed that active infection with HSV-2 in early pregnancy doubles the risk of developing autism in the male fetus. They found that increasing HSV-2 IgG levels in maternal mid-pregnancy plasma from 240 to 640 arbitrary units/mL doubles the risk of developing autism in male fetuses after adjusting for parity and the child's birth year. There were few female fetuses included in the study to conclude similar results. The presence of high mid-pregnancy antibody levels against HSV-2 might indicate an active maternal infection during early pregnancy[100]. However, a study by Gentile *et al*[101] showed no significant differences in the seropositivity rates and levels of anti-HSV-1 or anti-HSV-2 between children with autism and the controls. Another study by Slawinski *et al*[88] showed that the antenatal infection with CMV and not HSV-2 increases the risk of autism in the coming children. We still need more research to prove or disprove the effect of antenatal infection with HSV-2 on the risk of developing autism in the offspring.

Varicella-zoster virus: Varicella-zoster virus is s an alpha herpes DNA virus known as human herpesvirus 3 that causes chickenpox and herpes zoster (shingles). It is a neurotropic virus that can cause latent infection in the sensory nerve ganglia. It also induces neuroinflammation and is implicated in many central nervous system disorders, such as multiple sclerosis, that can share some common pathophysiologic features with ASD[102]. There is not much evidence about its role in the pathogenesis of autism. Gentile *et al*[103] showed an increased seropositivity rate for Varicella Zoster Virus in children with autism than in controls. They hypothesized that exposure to Varicella Zoster Virus and high titers of specific anti- Varicella Zoster Virus antibodies had a significant association with ASD. However, this association could be a cause or, on the other side, be due to increased susceptibility to infection with Varicella Zoster Virus due to vaccinophobia to MMR. Therefore, we need more studies to prove or disprove its causal relation. Some anti-varicella medications that are used to prevent or treat latent varicella infection can improve the symptoms of autism, establishing the causal relation of varicella infection with autism[104].

Influenza viruses: Influenza is caused by a group of enveloped negative-sense RNA Orthomyxoviridae viruses categorized into four main subgroups; Influenza types A, B, C, and D; among them, types A and B cause the well-known respiratory influenza disease. Influenza affects about 5%-15% of the world's population yearly, with more than three million people encountering severe infections and large numbers of hospitalization and deaths[105]. Influenza is associated with increased morbidity and mortality in high-risk groups, including pregnant women, and during the first two postpartum weeks, reliant on pre-existing immunity[106]. Some studies showed an increased risk of adverse neurodevelopmental outcomes such as autism or schizophrenia when pregnant ladies encounter influenza during pregnancy[107]. Mahic *et al*[108] found a statistically non-significant increase in the risk of autism in the offspring of seropositive mothers with symptoms of influenza during mid-pregnancy compared to

seronegative mothers. The chance may be responsible for this difference. Other biological factors, such as maternal immune system activation, could also be responsible. In addition, the influenza virus can trigger a cascade of acute-phase reactions, including fever which by itself increases the risk of autism, together with a systemic increase in cytokine expression[109].

However, many recent studies showed no increased risk of developing autism with influenza infection or vaccination during pregnancy in the offspring. In addition, Zerbo *et al*[110] showed no significant association between influenza infection and the risk of autism in the offspring. Meanwhile, they found a statistically non-significant increased risk of autism in the offspring when pregnant ladies received influenza vaccination during the first trimester. However, they emphasized that this observation should not call for any change in vaccination policy or practice. A more recent study by Becerra-Culqui *et al*[111] reported the same finding when they found no association between prenatal influenza infection or vaccination and increased risk of autism in offspring. They strongly recommended influenza vaccination to pregnant ladies to protect themself and their offspring. In addition, treatment of or prophylaxis against influenza during pregnancy did not pose a significant risk of autism. We should not ignore that influenza infection during pregnancy could increase the rate of complications such as preterm labor and encountering high-grade fever, which by itself increases the risk of autism. In addition, the discrepancies in the results of different studies could be related to methodological or population differences or the changes in the influenza virus structures across generations. Therefore, we need to perform more analyses on a broad scale.

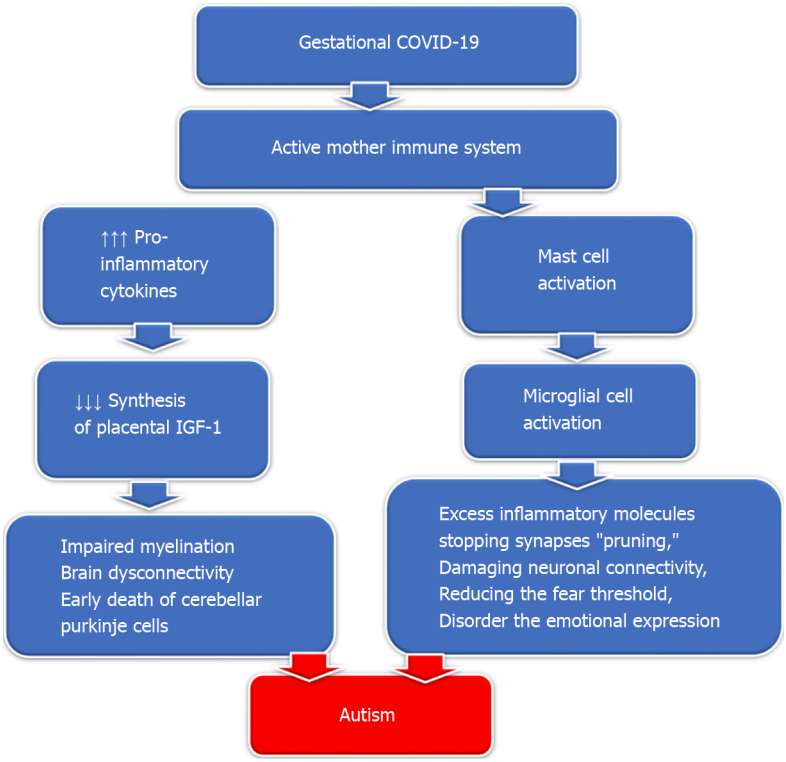
SARS-CoV-2 viruses: COVID-19 is caused by infection by SARS-CoV-2, one of the beta coronaviruses, which caused the pandemic coronavirus COVID-19, posing a severe threat worldwide. SARS-CoV-2 can spread from the respiratory tract to the central nervous system through the olfactory bulb. COVID-19 induces brain structural changes that cause various neurologic complications that could last long[112]. There is a rising concern about the potentially harmful effects of SARS-CoV-2 on pregnancy that could affect the pregnant lady and her fetus[113]. Currently, there is no proof of SARS-CoV-2 vertical transmission from the mother to her fetus, which could be due to the preventive effect of placental lactoferrin. However, the virus could be transferred postnatally *via* the mother's air droplets or during breastfeeding[114]. Severe gestational COVID-19 induces uncontrolled inflammatory cytokine storm release and maternal immune activation, causing possible fetal organ damage, including the brain, that could manifest later with autism symptoms[28]. The induced inflammation causes amygdala neurodegenerative changes by "short-circuiting the electrical system," producing emotional feeling ability impairment and abnormal fear regulation due to an abnormal hypothalamic-pituitary-adrenal axis system[115].

Gestational COVID-19 activates the maternal immune system, increasing the pro-inflammatory cytokine production that inhibits the synthesis of placental Insulin-like growth factor-1 (IGF-1). Decreased IGF-1 production impairs perinatal myelination and induces dysconnectivity of the developing brain with permanent neurological deficits[116]. IGF-1 deficiency causes reduced perinatal neo-neuronal myelination mediated by oligodendrocytes in the developing nervous system, which in turn causes the early death of cerebellar Purkinje cells and the development of autism[117]. Additionally, SARS-CoV-2 infection can activate mast cells which in sequence activate microglial cells, releasing excessive inflammatory molecules, stopping synapses "pruning," damaging neuronal connectivity, and reducing the fear threshold, disorderly the emotional expression detected in children with autism[118] (Figure 4).

These changes can explain the increase in the rate of autism during the COVID-19 pandemic. Edlow *et al*[119] showed that in-utero exposure to SARS-CoV-2 infection might be associated with increased neurodevelopmental disorder rates in some offspring. However, Brynge *et al*[120] showed that the increased association of autism and other intellectual disabilities in the offsprings of mothers infected with SARS-CoV-2 during pregnancy does not necessarily reflect the causal relationship but is more probable to be related to common familial conditions such as shared genetic and environmental factors. On the other hand, Dutheil *et al*[121] claimed that the COVID-19 pandemic might be a reason for a decrease in the incidence of autism in a paradoxical phenomenon due to decreased global air pollution, which is a significant environmental trigger for autism.

To lessen the influence of gestational COVID-19, pregnant women should have adequate amounts of n-3 polyunsaturated fatty acids, vitamin D, folic acid, and a high choline and luteolin supplement. These supplements benefit brain development and function in the offspring of women who encounter viral infections during early pregnancy. Luteolin is a potent natural flavonoid inhibitor of microglia and mast cell activation and prevents SARS-CoV-2 binding to ACE2 receptors[118,122].

Zika virus: Zika virus (ZIKV) is an anthropoid-borne positive-sense RNA Flavivirus that spreads mainly by biting infected Aedes species mosquitos (Ae. aegypti and Ae. albopictus). It poses a significant threat to human health worldwide[123]. The severity of ZIKV infection ranges from mild influenza-like infection to severe conditions with neurological complications such as seizures and Guillain–Barré syndrome. Some strains of ZIKV can cross the placental barrier and infect cortical progenitor cells to promote cell death *via* enhancement of apoptosis and autophagy, producing severe congenital malformations (ZIKV Congenital Syndrome)[124]. ZIKV Congenital Syndrome includes



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Figure 4 The effect of gestational severe acute respiratory syndrome coronavirus 2 infection on the offspring brain. Maternal infection with severe acute respiratory syndrome coronavirus 2 activates the mother's immune system, releasing an excess of pro-inflammatory cytokines that decreases the placental synthesis of IGF-1, resulting in impaired myelination and brain dysconnectivity and early death of cerebellar Purkinje fibres. In addition, maternal immune activation causes mast cell activation, which activates microglial cell activation. Microglial cell activation causes excess inflammatory molecules, stopping synapses "pruning," damaging neuronal connectivity, reducing the fear threshold, and impairing emotional expression ending in the development of autism manifestations. COVID-19: Coronavirus disease 2019.

microcephaly, hypoplasia or atrophy of the cerebral cortex, cerebellum, brainstem, abnormal cortical formation, corpus callosum anomaly, other neurological abnormalities, cerebral palsy, severe developmental delay, and eye defects, which are one of the severe complications that occur when the mother gets infected during pregnancy[125]. Unfortunately, no specific antiviral medications or vaccines are available against ZIKV infection[126].

In a large population-based mother-child cohort study during the 2016-ZIKV outbreak in the United States, about 15.3% of toddlers with in-utero exposure to ZIKV had abnormal neurodevelopment findings at two years of age[127]. The inflammatory process generated by ZIKV during pregnancy has bi-mutual pathways. In one aspect, it helps to eradicate the virus, and in another aspect, it causes damage to the developing brain. The released inflammatory mediators, such as interleukin-6 and tumour necrosis factor-alpha, affect brain development, delay neuronal maturation, alter brain connectivity, and trigger autism symptoms[128]. There were some reports of increasing reported cases of autism in the offspring of mothers infected with ZIKV during pregnancy. Nielsen-Saines *et al*[129] observed 18 children who developed neurological symptoms out of 216 offspring from mothers infected with ZIKV during pregnancy. Six children out of 18 had autism manifestations. In addition, three children born asymptomatic developed autism manifestations at one year of age. In addition, Abtibol-Bernardino *et al*[130] showed that five children out of 26 who had antenatal exposure to ZIKV developed severe neurological disorders; two of them had autism. Therefore, children with a positive history of in-utero ZIKV exposure should have a serial follow-up for any neurological impairment, including autism, even when born asymptomatic.

ANTIVIRALS AND DRUGS WITH ANTI-AUTISM EFFECTS

Henderson[131] described a four-year-old child with a bipolar disorder associated with violent aggression and manifestations of autism and attention deficit hyperactivity disorder resistant to conventional treatment, including anticonvulsants, methylphenidate, guanfacine, lithium, and neuroleptics. After the failure of multiple regimens to stabilize the mood, the patient received a trial of valacyclovir 1000 mg twice till improved, then with half the dose to complete three years. The patient improved

dramatically in irritability, volatile mood, social reciprocity, concentration, and overall personality. The potential benefit of valacyclovir could be related to improving subclinical herpes simplex. Meanwhile, Kimberlin *et al*[132] found that oral acyclovir (300 mg/square meter/dose taken three times daily for six months a) can improve the neurodevelopmental outcomes in children who survived neonatal herpes simplex disease with central nervous system involvement. In addition, naltrexone therapy may benefit children with autism, particularly in the presence of self-injurious behaviour, failure of other treatments, and "high opioid tone" autism[133]. Naltrexone has immune-modulating effects and prevents the activation of grey and white matter astrocytes in specific brain areas, especially frontal cortical tissues, causing a reduction of injurious behaviour[134]. Intravenous immunoglobulins (IVIG) are one of the well-established treatment methods for severe systemic infections, including viral infections[135]. IVIG showed promising efficacy in treating autoimmune encephalopathy in children with autism, especially with high anti-dopamine D2L receptor antibodies[136]. Connery *et al*[137] showed that IVIG effectively alleviated the symptoms of irritability in children with autism who developed autoimmune encephalopathy. The presence of anti-dopamine D2L receptor antibodies is associated with improved responsiveness to IVIG therapy with modulation of behaviour. Therefore, we can use these antibodies to predict the responsiveness to IVIC therapy in these children.

N-acetylcysteine is derived from the amino acid L-cysteine and helps to regenerate glutathione with the release of glutamate into the extracellular space, reducing glutamatergic neurotransmission at synapses, correcting brain glutaminergic dysregulation, and consequently improving the autistic manifestations. It helps to reduce irritability and hyperactivity and improves social awareness in patients with autism[138,139]. Boris *et al*[140] found in a small cohort of children with autism that oral using 30 or 60 mg of Pioglitazone for three to four months might induce apparent clinical improvement of the behavioural symptoms in those children without significant side effects. Pioglitazone is an antidiabetic medication that modulates the effect of insulin. Pioglitazone can reduce IL4 production in CD4 cells and block IL10 and IL4 production by T cells. It also may help shift the T-cell response from Th2 to Th1 or decrease Th2 cytokine expression. Therefore, oral Pioglitazone may be of therapeutic advantage in children with autism[141].

INCREASED RISK OF VIRAL INFECTIONS IN PATIENTS WITH AUTISM

Children with autism have more chances of getting infected. Sabourin *et al*[142] showed that children with autism are more susceptible to infections in the first four postnatal weeks and the first three years of life than the typically developed children. In addition, they found that children with autism also have more infections in the first four weeks of life than children with other developmental disorders. Children with autism have multiple co-morbidities and behavioural problems that increase the risk of contracting various infections, including viruses[97]. Table 2 shows some important risk factors and co-morbidities that increase the risk of contracting diseases in children with autism.

Children with autism have a high rate of autoimmune diseases, immune dysregulation, and impaired levels of immune mediators, indicating continuing immune dysfunction[51]. Some genetic causes of autism are associated with an immune deficiency that increases the risk of infections, including viral disease, *e.g.*, Timothy syndrome[143]. Heuer *et al*[144] showed that children with autism have lower plasma IgG and IgM levels than typically developed children and children with developmental delays. The levels of these immunoglobulins were negatively correlated with the autism severity, as indicated by the Aberrant Behavior Checklist score. Children with autism commonly have mitochondrial dysfunction. Mitochondrial dysfunction is associated with reduced oxidative phosphorylation and burst in granulocytes which causes an impaired immune response and weak antioxidant defence[145].

Children with autism are subject to restrictive nutrition supplements either due to the restrictive and selective behaviour associated with autism, sensory-based feeding problems, relational sphere disorders, biological food intolerance, medically related feeding problems, and restrictive dietary management, that expose them to various nutritional deficiencies which further impair their immunity and susceptibility to infection[97]. Babaknejad *et al*[146] found statistically significantly lower plasma zinc levels in patients with autism than in healthy controls. Zinc deficiency negatively impairs children's mental development and physical growth and might damage their immune systems[147]. Vitamin C deficiency was also reported in some patients with autism due to the severely restricted diet or the associated gastrointestinal disorders that prevent adequate vitamin C intake or absorption[148,149]. Vitamin C is essential for the integrity of the mucous membrane and general immunity[147]. In addition, children with autism have the highest rate of vitamin A deficiency compared to other micronutrients, and the degree of deficiency is correlated with autism severity[150]. Vitamin A significantly impacts gastrointestinal functions and the skin's innate immunity. Therefore, vitamin A deficiency increases the risk of skin infections[151]. Vitamins A and D regulate microbial complexity, mucosal barrier function, and immune responses, ensuring intestinal homeostasis. Therefore, its deficiency induces gut dysbiosis and enhances the dysbiotic microbial communities, increasing susceptibility to infection and the risk of gastrointestinal tract injury[152].

Table 2 Important risk factors and co-morbidities that increase the risk of contracting infections in children with autism

Risk factors	Description
Immune system disorders	High rate of autoimmune diseases
	Immune dysregulation of T cell functions
	Impaired levels of immune mediators
	lower plasma IgG, and IgM
	Continuing immune dysfunction
	High rate of mitochondrial dysfunction
	Oxidative stress
	Neutrophils dysfunction
Medical co-morbidities	Genetic disorders: *e.g.*, fragile X syndrome, Down syndrome, Duchenne muscular dystrophy, tuberous sclerosis complex, and neurofibromatosis type I
	Neurological disorders: *e.g.*, cerebral palsy and congenital abnormalities
	Gastrointestinal disorders: Gastroesophageal reflux and inflammatory bowel disease
	Metabolic disorders: mitochondrial disorders, disorders of creatine metabolism, selected amino acid disorders, disorders of folate or vitamin B12 metabolism, and selected lysosomal storage disorders
	Allergic disorders: Such as asthma, nasal allergies, atopic diseases (IgE-mediated)
Behavioral problems	Stereotyping behavior: Affecting mouth and general hygiene
	Mouthing and pica behavior
	Faecal smearing
Feeding and nutritional disorders	Biological food intolerance
	Restrictive and selective behavior
	Sensory-based feeding problems
	Relational sphere disorders
	Medically-related feeding problems
	restrictive dietary management
	Nutritional deficiencies: *e.g.*, Vitamin A, D, C, and zinc
Gastrointestinal dysfunctions	Gastroesophageal reflux
	Autonomic dysfunction and Impaired intestinal motility
	Gastric hypoacidity and
	Impaired digestive enzyme production
	Gut dysbiosis
	Inflammatory bowel disease
Vaccinophobia	Lack of parental education and awareness
	Anti-vaccine movement

Normal intestinal motility is required for healthy gut microbiota by washing out any abnormal bacterial growth in the body. Children with autism have impaired intestinal peristalsis and digestive abilities due to autonomic dysfunction. They frequently get constipation resulting in abnormal proliferation and impaired clearance of the pathogenic intestinal bacteria. In addition, children with autism have gastric hypoacidity and impaired digestive enzyme production, which augments the resulting dysbiosis[153]. Conversely, gut dysbiosis correlates with the severity of autism, cytokine quantities, and tryptophan homeostasis. Therefore, gut microbiota modulation may alleviate the symptoms of autism [154].

Another critical risk factor that increases the risk of viral infections in children with autism is the reluctance of some parents to vaccinate their children with autism. Despite the marked progress in studying the epidemiology, aetiology, pathophysiology, and genetics of autism; many misguided scientists; politicians, and frustrated parent groups still resist vaccinating children with autism[155]. Since Andrew Wakefield and his 12 colleagues published their frauded paper in British Medical Journal

in 1998, the vaccination rate of children with autism significantly declined, especially for measles, mumps, and rubella[156]. The resultant antivaccine movements considered three main pillars for their action. They hypothesized that combining the measles-mumps-rubella vaccine can induce intestinal mucosal damage, allowing the entry of encephalopathic proteins that trigger autism. In addition, they suppose that the ethylmercury-containing vaccine preservative thimerosal is neurotoxic. They also suggest that concurrently administering multiple vaccines devastates or weakens the immune system [157]. Zerbo *et al*[158] found that the vaccination rate in children with autism and their younger siblings was significantly lower than in the typically developed children. Parents of children with autism were more reluctant to vaccinate at least one recommended vaccine for the child's younger sibling and to limit the number of vaccines given during the first year of life of the younger siblings.

Limitations and future direction

Many included studies were of a limited number of patients or were done in animal models. We need to have long-term, multicentered studies that include different races and populations to better judge the interplay between infections and autism as a cause or effect. Pregnant women should ensure their vaccination against influenza, as this can prevent them from getting the flu while pregnant, which can cause complications for the mother and the baby[105-108]. When women get vaccinated against influenza, they protect their unborn babies from getting this illness while in the womb. Studies have shown that influenza virus infection can cause severe problems for babies if infected during the first trimester of pregnancy, leading to preterm birth or stillbirth in some cases[109-111]. By getting vaccinated, pregnant women can protect themselves and their unborn babies from the flu while helping to reduce the spread of the disease in the community. Pregnant women could have enough vitamin D, folic acid, n-3 polyunsaturated fatty acids, and high choline and luteolin[117,122].

Early screening and possibly diagnosis to detect and may prevent autism are crucial for reducing the burden of this condition. Long-term follow-up is necessary for infants whose mothers report an inflammatory event due to viral infection at any time during pregnancy to monitor for signs of autism. In addition, children with autism should be screened for congenital rubella and cytomegalovirus infections [76,96]. Further research is needed to investigate whether specific vaccines or other measures taken during pregnancy can prevent the development of autism in children born to mothers whose certain viruses have infected them.

CONCLUSION

Autism is a group of heterogeneous, multi-factorial, neurodevelopmental disorders. Several factors play together to increase the risk of development of autism, including genetic, epigenetic, and environmental factors, together with antenatal and early-life infections. Viral infection during critical periods of early in-utero neurodevelopment may lead to an increased risk of autism. Maternal infection by DNA or RNA viruses that can cross the maternal-fetal interface may induce neurodevelopmental problems in the developing fetus. Viral infection induces neuroinflammation that affects many neurological processes, including neural development, brain structures, synapse plasticity, cognition, and behaviour; therefore, it may end in the development of autism. There are many shreds of evidence that rubella, cytomegalovirus, herpes simplex virus, influenza viruses, SARS-CoV-2 viruses, and zika virus infections during pregnancy and early life can trigger autism development. Conversely, children with autism are at increased risk of infections, including viruses. Every effort should be made to prevent maternal and early-life infections and reduce the risk of autism. Vaccination against common viral agents may help to reduce the prevalence of autism. Immune modulation of children with autism should be considered to reduce the risk of infection.

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MINIREVIEWS

Pediatric multisystem inflammatory syndrome associated with COVID-19: Insights in pathogenesis and clinical management

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Abstract

The pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has been a major challenge to be faced in recent years. While adults suffered the highest morbidity and mortality rates of coronavirus disease 2019, children were thought to be exclusively asymptomatic or to present with mild conditions. However, around April 2020, there was an outbreak of a new clinical syndrome related to SARS-CoV-2 in children - multisystemic inflammatory syndrome in children (MIS-C) - which comprises a severe and uncontrolled hyperinflammatory response with multiorgan involvement. The Centers for Disease Control and Prevention considers a suspected case of MIS-C an individual aged < 21 years presenting with fever, high inflammatory markers levels, and evidence of clinically severe illness, with multisystem (> 2) organ involvement, no alternative plausible diagnoses, and positive for recent SARS-CoV-2 infection. Despite its severity, there are no definitive disease management guidelines for this condition. Conversely, the complex pathogenesis of MIS-C is still not completely understood, although it seems to rely upon immune dysregulation. Hence, in this study, we aim to bring together current evidence regarding the pathogenic mechanisms of MIS-C, clinical picture and management, in order to provide insights for clinical practice and implications for future research directions.

Key Words: Pediatric multisystem inflammatory disease; COVID-19 related; Multisystem inflammatory syndrome in children; COVID-19; SARS-CoV-2; Etiology; Disease management

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Core Tip: Multisystem inflammatory syndrome in children (MIS-C) comprises a severe and out-of-control inflammatory response with multiorgan dysfunction following severe acute respiratory syndrome coronavirus 2 infection. Despite its severity, there are no definitive disease management guidelines for this condition. Conversely, the complex pathogenesis of MIS-C is still not completely understood, although it seems to rely upon immune dysregulation. Hence, in this study, we aim to bring together current evidence regarding the pathogenic mechanisms of MIS-C, clinical picture and management, in order to provide insights for clinical practice and implications for future research directions.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) pandemic has been a major challenge to be faced in recent years. With variable clinical manifestations, the disease mainly affects older people with comorbidities, whereas children were not initially considered a risk group for its severe form[1]. However, even though COVID-19 in children is generally milder when compared to adults, it was noticed that a small part of infected children are subject to a post-infectious severe condition presenting with multiorgan dysfunction and systemic inflammation, thus called multisystem inflammatory syndrome in children (MIS-C) associated with COVID-19[2,3]. Several aspects of this syndrome are still unclear, given its recent discovery. Initially, the heterogeneous clinical picture of MIS-C was attributed to conditions such as Kawasaki disease (KD) or toxic shock syndrome, due to the existing similarities. However, as COVID-19 pandemic progressed, there was an urge to recognize MIS-C as a new clinical condition that needs to be thoroughly understood[4].

Epidemiological data, although not very robust, suggest that the incidence of MIS-C is higher in African, Afro-Caribbean and Hispanic countries, while the incidence in East Asian countries is lower[5]. Furthermore, factors such as age, viral load and chronic comorbidities are considered risk factors for severe manifestations of COVID-19 and therefore may be associated with the development of MIS-C[6-8].

MIS-C usually develops weeks after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and, even though its pathophysiological mechanisms are still unclear, studies point to an exacerbated immune response that leads to a state of hyperinflammation, *i.e.*, an imbalance between host pro-inflammatory and anti-inflammatory mechanisms with the potential to affect multiple organs [9]. Regarding its presentation, MIS-C associated with COVID-19 has a broad clinical spectrum in which fever is the main symptom. In addition, patients may also manifest gastrointestinal disorders, such as diarrhea and vomiting, abdominal pain, shock and/or hypotension, respiratory symptoms such as cough and dyspnea, as well as cardiac and neurological symptoms[10].

There are differences between the main definitions of MIS-C made by the Royal College of Pediatrics and Child Health (RCPCH), Center for Disease Control and Prevention (CDC) and the World Health Organization (WHO). However, all three institutions agree on the presence of fever, laboratory findings indicative of active inflammation, multisystem organ involvement without a plausible underlying diagnosis, as well as proven infection by COVID-19 or recent exposure to a case of COVID-19[11]. In this sense, a well-established definition is essential for early diagnosis and exclusion of the main differential diagnoses.

Considering that MIS-C is a serious condition with numerous possibilities of complications and scarce scientific evidence on the subject, standardizing a therapeutic line to be followed has been a challenge[5]. The main guidelines advise that the treatment should be done individually, aiming to control hyperinflammation and recover organic function[12]. Some medications such as intravenous immunoglobulin (IVIG) and other anti-inflammatory and immunosuppressive therapies are considered the mainstay of treatment for MIS-C. In addition, other drugs such as steroids and immunobiologicals and aspirin have also been widely used with reservations[13]. This minireview aims to bring together current evidence regarding the pathogenic mechanisms of MIS-C, clinical picture and management, in order to provide insights for clinical practice and implications for future research directions.

PATHOGENESIS OF MIS-C

The pathogenesis of MIS-C is a complex event that has not yet been fully elucidated. To date, three hypotheses that may be associated with the development of the syndrome are highlighted: (1) SARS-CoV-2 spike protein superantigenic profile[14,15]; (2) Chronic response to viral antigen exposure[16]; or (3) Production of autoantibodies in response to the viral infection[17]. These may provide a subset for further understanding about the immunopathological features of MIS-C, which, in turn, apparently rely on autoreactivity, cytokine storm, and immune dysregulation[18]. Thus, we hereby discuss, in detail, the main findings regarding the immunological issues associated with MIS-C.

Immune dysregulation

A major factor that maintains the hyperinflammatory state of MIS-C is immune dysregulation[19]. Currently, several manuscripts have demonstrated the occurrence of disorders related to innate and adaptive immunity and to cytokine response[20-22]. In this regard, Huang *et al*[20] exploit some of these aspects. Apparently, classic monocytes and type 2 dendritic cells were found to be downregulated in MIS-C, while type 1 DCs were greatly activated. Interestingly, some findings also suggest that this syndrome presents with impaired antigen presentation, given the low levels of HLA-DR and CD86 in monocytes and dendritic cells[23]. Altogether, these data support a possible role for antigenic cross-presentation as one of the possible mechanisms of MIS-C, since this process is strongly related to the action of CLEC9A, a major marker of type 1 DCs[20]. Moreover, elevated expression of alarmin-related S100A genes is also documented[19]. These molecules are closely related to the triggering of inflammatory mechanisms in the innate immune response, *via* Toll-like receptors (TLRs), and play a role in apoptosis and organ damage[24].

As for T cells, Ramaswamy *et al*[19] describe an increased expression of perforins, granzyme A and H on natural killer cells and CD8+ T cells. These cytotoxic molecules greatly increase tissue damage characteristic of MIS-C. Also, skewed T cell receptor (TCR) repertoire with TRBV11-2 expansion, found in severe MIS-C patients may indicate exposure to a superantigen (SAg) or be related to the presence of autoreactive T cells, while γδ and CD4+CCR7+ T cells activation is highlighted by elevated HLA-DR expression[23]. On the other hand, B-cells show an intriguing pattern in MIS-C, characterized by increased numbers of plasmablasts. However, their role in the pathophysiology of the syndrome requires further investigation[25]. Additionally, MIS-C patients apparently present interferon-gamma (IFN-γ) response dysregulation, which is characterized by exacerbated production of CXCL9 in response to IFN-γ[22]. Of note, excessive production of this chemokine is related to increased disease severity[26].

Cytokine storm

Emerging evidence has highlighted MIS-C as a state of out-of-control release of cytokines - referred to as "cytokine storm" - and immune cell hyperactivation[27]. In fact, high levels of interleukin-1β (IL-1β), IL-6, IL-8, IL-10, IL-17, and IFN-γ, with raised C-reactive protein (CRP) and ferritin, were reported in the acute phase of MIS-C[23]. Carter *et al*[23] also demonstrated that MIS-C patients show raised fibrinogen levels, raised D-dimer, and low platelet count, which suggests a possible interplay between cytokine storm/hyperinflammation and pro-coagulant state development. This crosstalk has also been extensively explored in the pathophysiology of severe acute COVID-19[28-30]. Since the up-regulated cytokines IL-1β, IL-6, and IL-8 are critically involved in pro-inflammatory-induced coagulation in other conditions, their role in MIS-C-related hypercoagulable state should be further investigated[31-34].

Except for IL-1β and IL-17 elevations, these results are ratified by Diorio *et al*[35]. In that study, the authors report that the sum of IL-10 and tumor necrosis factor (TNF)-α levels was hypothesized to differentiate between MIS-C and severe COVID-19 patients [mean (95% confidence interval); severe: 30.06 (9.54-50.6) *vs* MIS-C: 82.25 (32.5-132.0), $P = 0.036$]. The significant and paradoxical elevation of IL-10 - a potent anti-inflammatory cytokine with immunoregulatory functions[36] - also differs from the cytokine profile seen in KD[37]. There were also noted increases in markers of endothelial dysfunction in both MIS-C and severe COVID-19 with elevated sC5b-9 concentrations. At last, univariate linear regression modeling revealed that sC5b-9 levels correlated in a statistically significant manner with IL-6, IL-8, and TNF-α[35].

Although Diorio *et al*[35] demonstrate that sC5b-9 was significantly elevated in patients with severe COVID-19 in comparison with those with minimal COVID-19, the observed elevated levels in MIS-C patients did not reach statistical significance. However, a subsequent study by Syrimi *et al*[21] revealed that complement protein C9 and C5b-9 were significantly increased in MIS-C patients at the acute stage of the disease. C5b-9 is a marker of the formation of the membrane attack complex, the final common pathway of complement activation[38]. The hyperactivation of complement components including C5a in sera and C5b-9 correlates with the out-of-control release of pro-inflammatory cytokines including TNF-α, IL-1, and IL-6[39]. These authors also demonstrated that MIS-C patients had significantly increased levels of the chemokine's monocyte chemoattractant protein 1 (MCP-1/CCL2) and interferon gamma-induced protein 10 (IP-10/CXCL10). Higher levels of IL-6, IL-18, and IL-10 were also observed [21]. On the other hand, in contrast with previous findings, no difference was observed in the levels of

IL-1β, IL-8 (CXCL8), IL-17A, IFN-α_2, IFN-γ, and TNF-α in MIS-C patients in comparison with the healthy donor controls.

Cytokine profiling also identified elevated signatures of inflammation (IL-18 and IL-6), lymphocytic and myeloid chemotaxis and activation (CCL3, CCL4, and CDCP1), and mucosal immune dysregulation (IL-17A, CCL20, and CCL28) in another report[40]. Gruber *et al*[40] suggested that elevations in unique chemokines (CXCL5, CXCL11, CXCL1, and CXCL6) and cytokines (including IL-17A, CD40, and IL-6) appear to distinguish MIS-C patients from pediatric COVID-19 patients. In another study, IL-6, IL-17A, and CXCL10 were also found to contribute the most to the cytokine storm. In contrast, the main negative contributors were adenosine deaminase, stem cell factor, and TWEAK[17] - a suppressor of production of IFN-γ and IL-12, that attenuates the innate response and its transition to adaptive Th1 immunity[41]. However, despite being raised in MIS-C patients, IL-17A seems to drive Kawasaki- but not MIS-C-associated hyperinflammation[17].

At last, more recent studies reiterate that a robust cytokine release might explain the severity and outcome of MIS-C. Abo-Haded *et al*[42] recently reported that mild MIS-C patients present higher levels of IFN-α, IFN-γ, IL-6, IL-8, IL-10, and CXCL10 in comparison with the control group. Conversely, there was an extremely significant difference regarding all measured cytokines when comparing both study groups (mild and severe) ($P < 0.0001$)[42]. Severe MIS-C patients showed higher levels of IFN-α, IL-1β, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), and HMGB1 ($P < 0.0001$), alongside less significant increases ($P < 0.05$) in IL-8, TNF, and GM-CSF. These findings suggest that the cytokine profile and its serum levels may be determining factors for the clinical outcome of patients with MIS-C.

Cytokine storm-induction mechanism

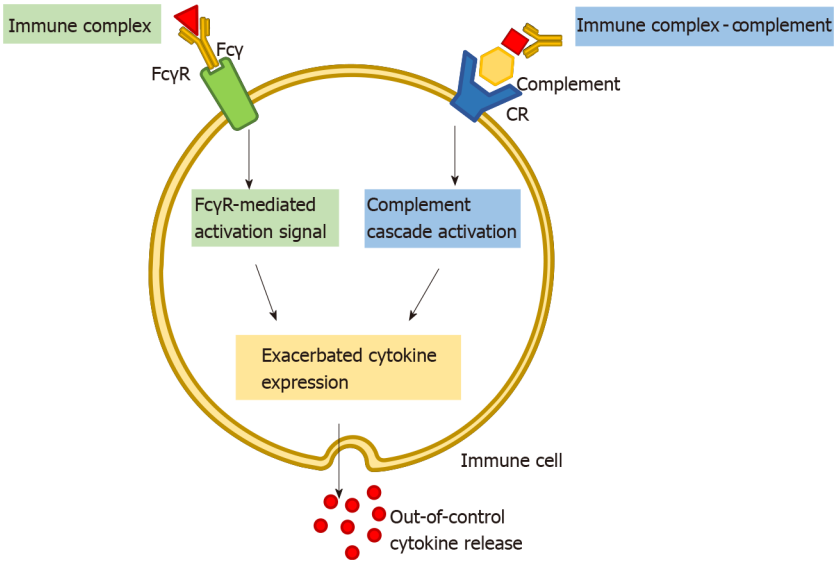
Despite these findings, the molecular features of the MIS-C-related cytokine storm have not been completely understood. One potential explanation for the out-of-control release of cytokines seems to be an antibody-dependent enhancement (ADE) mechanism[43,44]. In ADE - also called immune enhancement - pre-existing non-neutralizing or sub-neutralizing antibodies (nNAbs) bind to virus-derived antigens[43,44]. The resulting immunocomplexes may further interact with the immune cell's membrane harboring the immunoglobulin G (IgG) Fcγ receptor (FcγR) through the Fc domain of the immunoglobulin, thereby activating the immunoreceptor tyrosine-based activation motifs of these receptors[45-47]. When triggered, downstream signaling pathways induce the release of a variety of cytokines and immune cell recruitment[48,49]. The engagement of complement receptors could also represent a possible ADE-related mechanism. Once recognized by complement receptors, immunocomplexes bonded to complement-derived molecules could also trigger cytokine release and immune activation[49] (Figure 1). On the other hand, ADE may also promote SARS-CoV-2 epitope intake into the host cell cytoplasm[50]. nNAbs-antigen immunocomplexes interaction with FcγR facilitates the internalization of the virus into host cells by endocytosis[48]. At last, upon internalization, endocytic TLRs can then recognize these viral particles and also induce immune cell activation and recruitment[50].

In contrast, other studies have highlighted that MIS-C cytokine storm could also be attributed to a SAg-induced immune response. SAgs are highly potent immunostimulatory molecules that induce T cell activation through binding to specific β chains of TCRs at their variable domain in a complementarity-determining region 3-independent (CDR3-independent) manner[51,52]. This activation requires simultaneous interaction of the SAg with the Vβ domain of the TCR and HLA class II molecules on the surface of an antigen-presenting cell[53]. The specificity of SAg attachment to different TCR Vβ chains results in Vβ skewing, whereby the frequency of T cells responding to SAg exposure exceeds that of conventional peptide antigens[54].

It has been recently demonstrated that an insertion unique to SARS-CoV-2 spike glycoprotein exhibits a SAg-like sequence motif that possesses a high affinity for binding TCR, interacting closely with both the α- and β-chains variable domains' complementarity-determining regions[14,55]. In this sense, Porritt *et al*[15] showed that MIS-C patients present a profound expansion of TCRβ variable gene 11-2 (TRBV11-2), with up to 24% of clonal T cell space occupied by TRBV11-2 T cells. The author also found a significant correlation between TRBV11-2 usage and TNF-α, IFN-γ, IL-6, and IL-10 levels[14]. Therefore, it seems that the TRBV11-2 expansion and possible activation may be one of the main features in the MIS-C-related out-of-control cytokine release. These findings were ratified by Moreews *et al*[56], that observed a specific expansion of activated T cells expressing the Vβ21.3 T cell receptor β chain variable region in both CD4 and CD8 subsets, and this was also associated with the observed cytokine storm. Although multiple cytokine storm-induction mechanisms have been proposed, further research is needed to build a more reliable model of MIS-C-related immunopathophysiology.

Autoreactivity

The rapid resolution of disease following high-dose IVIG administration has raised the hypothesis that MIS-C could be an autoantibody-mediated condition[57]. IVIG is a usual approach to triggering inhibitory FcγR, thus exerting a multitude of immunomodulatory properties[58]. Upon FcγR engagement, IVIG may thereby preclude Th1 and Th17 differentiation, enhance CD4+FoxP3+ regulatory T cells expansion, and inhibit autoantibody release by B-cells, for example[59-61].



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Figure 1 Simplified scheme of antibody-dependent enhancement mechanism of multisystemic inflammatory syndrome in children-C-related cytokine storm. FcγR: Fcγ receptor; CR: Complement receptor.

Based on this, Gruber *et al*[40] tested the hypothesis that SARS-CoV-2 infection leads to a secondary autoreactive humoral response. These authors' differential autoantibody analysis returned 189 peptide candidates for IgG autoantigens and 108 IgA autoantigens. More interestingly, the majority of these antigens present enrichment in organ systems that play a central role in the pathophysiology of MIS-C. Possible autoantigens include peptides expressed in the gastrointestinal tract (MUC15, TSPAN13, and SH3BP1), the endothelial and cardiac tissue (P2RX4, ECE1, and MMP14), and, notably, in immune cells (CD244, IL-1A, IFNGR2, IL-6R, and LAMP1)[40]. Moreover, the analysis of MIS-C plasma also revealed well-known disease-associated autoantibodies - namely anti-La, and anti-Jo-1[40]. Similarly, Consiglio *et al*[17] found that 26 Gene Ontology (GO)-terms were enriched in MIS-C samples. Among these, there were autoantigen peptides involved in lymphocyte activation processes, immune cell signalling, and structural proteins in the heart and blood vessels[17]. In turn, Porritt *et al*[61] identified a number of IgG-target tissue-specific antigens from the gastrointestinal and cardiovascular tracts, skeletal muscle, and brain tissues. On the other hand, Burbelo *et al*[62] have failed to detect autoantibodies against the majority (16/18) of the most relevant autoantigens raised by previous studies in MIS-C patients who did not receive IVIG. These data have highlighted that the first-line IVIG therapy may be a confounding factor in autoantibody measurements in MIS-C[62]. Therefore, these results suggest that secondary autoreactive humoral response may play a pivotal role in MIS-C pathobiology; however, further studies, taking into account possible confounding factors, are necessary to elucidate this matter.

CLINICAL MANAGEMENT

Clinical and laboratory features

Regarding the clinical manifestations of MIS-C, we relied on recent meta-analyses to identify the most prevalent symptoms of the syndrome. Overall, fever was the most predominant reported symptom, being present in 82.4% to 100% of total cases and with median duration of approximately 5 d, followed by gastrointestinal symptoms including diarrhea, abdominal pain, and vomiting (82%-85.2%)[10,63-66]. Respiratory symptoms have also been reported (25%-50.3%), such as cough, dyspnea, and sore throat, although not as prevalent as expected, which diverges from the classical COVID-19 manifestations, especially in comparison to adults[63,64]. Thus, a greater vulnerability for children to develop gastrointestinal symptoms is apparent, which highlights the need to consider the possibility of MIS-C in infants with non-respiratory manifestations along with a hyperinflammatory profile.

In addition, cutaneous manifestations similar to KD are reported, especially, polymorphic maculopapular exanthema (63.7%), and non-purulent conjunctivitis (56%)[65]. Furthermore, neurological and cardiac involvement are also outlined and represent a relevant aggravating factor. In this sense, neurological symptoms are commonly expressed as headaches, in most cases, irritability and lethargy [10,64,66]. On the other hand, cardiocirculatory manifestations are more frequently observed, present in approximately 80% of cases, with a broad spectrum that includes from tachycardia (76.7%), hypotension (77%) and shock (52%-68.1%) to myocarditis (41.4%), aneurysms (10.3%) and mild dilatation of the coronary artery (11.6%)[63,64,66].

Consistent with the syndromic manifestations of MIS-C, the laboratory findings demonstrate an expected hyperinflammatory state. The most important laboratory findings demonstrate increased levels of inflammatory markers, such as CRP, ferritin and IL-6[63,66], along with increased erythrocyte sedimentation rate and procalcitonin[66,67]. Moreover, complete blood count often reveals leukocytosis, with increased neutrophil levels[67]. Lymphocytopenia is also a common finding, along with anemia and normal or reduced platelet count[63,64,66]. Regarding the coagulation panel, D-dimer and fibrinogen showed a considerable increase[64,65,67].

As for markers of myocardial injury, such as troponin, B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP), evidence points to a frequent elevation, being current factors of concern in the management of children with MIS-C[63,64,67]. Troponin levels > 32 pg/mL are considered a major predictor of myocardial involvement in MIS-C[68]. Similarly, elevated levels of NT-proBNP are associated with myocardial dysfunction and severe MIS-C, being considered a useful biomarker for early identification of impaired cardiac function[69].

Diagnosis

Currently, MIS-C diagnosis is conducted through criteria established by important health institutions on characteristics such as age, clinical picture, inflammatory markers, and molecular or serological COVID-19 testing[70]. Of note, three distinct case definitions stand out in the diagnosis of MIS-C: That of the RCPCH[71], the WHO[72] and the CDC[73].

Overall, when faced with a suspected case of MIS-C, specific laboratory findings should be measured to assist the diagnosis[74]. In this regard, Gottlieb *et al*[75] points out the major parameters necessary for the evaluation of these cases, such as: Complete blood cell count; electrolytes; renal and liver function; inflammatory markers (CRP, D-dimer, albumin and coagulation panel); BNP; tests for SARS-CoV-2, such as polymerase chain reaction (PCR) and/or serologies; as well as troponin, ferritin, fibrinogen and procalcitonin.

In this context, the available case definitions serve as a guide for clinical practice, as they synthesize the main findings related to the diagnosis of MIS-C. According to the RCPCH, MIS-C is characterized by persistent fever > 38.5 °C, inflammation and single or multi-organ dysfunction, along with additional features such as abdominal pain, conjunctivitis, lymphadenopathy and rash and exclusion of any other microbial cause[71]. On the other hand, WHO case-definition of MIS-C includes individuals aged 0-19 years with fever ≥ 3 d, at least 2 concomitant systemic symptoms, elevated markers of inflammation, evidence of COVID-19 or contact with contaminated patients and no apparent other etiology for the inflammation[72]. Ultimately, CDC diagnosis of MIS-C includes: Individuals under 21 years of age presenting with fever ≥ 38.0 °C for ≥ 24 h; laboratory evidence of inflammation, presence of severe multisystemic disease; no other plausible alternative diagnosis; presence of reverse transcription PCR (RT-PCR), serology, or antigen test positive for COVID-19 currently or recently, or exposure to the virus within 4 wk prior to symptom onset[73]. Table 1 summarizes the major differences between these case definitions. Among these case definitions, Hoste *et al*[63] demonstrate a greater accuracy of the WHO MIS-C definition, comprising 97% of cases, followed by the CDC (62%) and lastly, the RCPCH.

Treatment

Treatment for patients with MIS-C relies on continuous monitoring of inflammatory markers to assess the progression of the condition[67]. Given the multisystemic involvement of MIS-C, its management should be performed with multidisciplinary team support, such as pediatric cardiologist, rheumatologist, and infectologist, and may require the assistance of other specialties, such as intensive care, neurology, nephrology, or gastroenterology[18]. Management of the condition follows a supportive approach and an immunomodulatory perspective. Thus, supportive care is crucial, including respiratory and circulatory support, ranging from intubation, intravenous fluids and inotropic support to extracorporeal membrane oxygenation[76]. In addition to supportive measures, immunomodulatory therapy with IVIG and steroids is established as first-line treatment for children with MIS-C, with a recommended dose of 2 g/kg IVIG[5,77]. In this regard, the American College of Rheumatology (ACR) prescribes IVIG alone in patients without shock or organ-threatening diseases, whereas the combination of IVIG combined with glucocorticoids in patients presenting with these conditions is recommended[5]. Furthermore, in patients in whom IVIG and corticosteroid therapy is not successful, the ACR recommends treatment with anakinra or infliximab at high doses[5].

Some studies have compared the efficacy of treatment with combined IVIG and methylprednisolone *vs* IVIG alone[78,79]. It is noted that the combined regimen is associated with significantly lower risk of poor outcome, such as treatment failure, acute myocardial dysfunction, need for hemodynamic support or second-line treatments. Furthermore, it is also established that in patients at high risk of thrombosis, anticoagulation therapy may be required[5], and that children with shock should be treated according to established guidelines for pediatric septic shock[80]. Given the complexity of management, children presenting with MIS-C-related symptoms should be quickly transferred to a pediatric intensive care center once stabilized[75].

Table 1 Case definition criteria for multisystemic inflammatory syndrome in children issued by the Royal College of Pediatrics and Child Health, World Health Organization and Centers for Disease Control and Prevention

Criteria	RCPCH[71]	WHO[72]	CDC[73]
Age	ND	< 21	< 19
Clinical	Persistent fever > 38.5 °C. Evidence of single or multi-organ dysfunction with additional features[1]	Fever ≥ 3 d; two of the following: (1) Mucocutaneous inflammation signs; (2) Hypotension or shock; (3) Cardiac issues[2]; (4) Evidence of coagulopathy; and (5) Acute gastrointestinal problems	Fever ≥ 38.0 °C for ≥ 24 h; multisystemic disease involving multiple (≥ 2) organs (cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic, or neurological). Requirement of hospitalization
Inflammatory markers	Neutrophilia, elevated CRP and lymphopenia	Elevated markers (such as ESR, CRP or procalcitonin)	Elevated CRP, ESR, fibrinogen, procalcitonin, d-dimer, ferritin, neutrophils, LDH, or IL-6 or reduced albumin and/or lymphocytes
COVID-19 test	Positive or negative SARS-CoV-2 RT-PCR test	Evidence of COVID-19 (*via* RT-PCR, antigen test, or serology) or probable contact with patients contaminated with SARS-CoV-2	RT-PCR, serology or antigen test positive for COVID-19 (currently or in recent period) or exposure to the virus within 4 wk prior to symptom onset
Exclusion factors	Exclusion of any other microbial cause	No other obvious etiology for the inflammation	Absence of other plausible alternative diagnoses

[1]Additional features: Abdominal pain, confusion, conjunctivitis, cough, diarrhoea, headache, lymphadenopathy, mucus membrane changes, neck swelling, rash, resp symptons, sore throat, swollen hands and feet, syncope, vomiting.
[2]Cardiac issues: Myocardial dysfunction, pericarditis, valvulitis, or coronary abnormalities (including ECHO findings or elevated Troponin/NT-proBNP).
RCPCH: Royal College of Paediatrics and Child Health; WHO: World Health Organization; CDC: Centers for Disease Control and Prevention; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; LDH: Lactate dehydrogenase; IL-6: Interleukin 6; RT-PCR: Reverse transcription-polymerase chain reaction; ND: Not described; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019.

CONCLUSION

In this study, we have brought together the current evidence regarding the pathogenic mechanisms of MIS-C, clinical picture and disease management. Emerging evidence has highlighted this condition as a state of out-of-control release of cytokines, immune cell recruitment and activation, and production of auto-reactive peptides. However, there is still much to be clarified regarding the mechanisms behind the induction of this response profile. Treatment for patients with MIS-C currently relies on continuous monitoring of inflammatory markers to assess the progression of the condition and IVIG administration. However, despite MIS-C severity, there are still no definitive disease management guidelines for this condition.

FOOTNOTES

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MINIREVIEWS

Etiopathogenic theories about long COVID

Luis Del Carpio-Orantes

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Abstract

The main etiopathogenic theories of long coronavirus disease (COVID) are listed and a conjunction of them is carried out with the objective of deciphering the pathophysiology of the entity, finally the main lines of treatment existing in real life are discussed (Paxlovid, use of antibiotics in dysbiosis, triple anticoagulant therapy, temelimab).

Key Words: Long COVID; Viral persistence; Amyloid microthrombosis; Dysbiosis; Weakened immune system; Paxlovid; Triple therapy

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Core Tip: List the main etiopathogenic theories of long coronavirus disease (COVID) and evaluate their interrelation as pathophysiological agents of long COVID syndrome.

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INTRODUCTION

Long coronavirus disease (COVID) is broadly defined as signs, symptoms, and conditions that continue or develop after initial COVID-19 or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The signs, symptoms, and conditions are present four weeks or more after the initial phase of infection; may be multi-systemic; and may present with a relapsing– remitting pattern and progression or worsening over time, with the possibility of severe and life-threatening events even

months or years after infection; this being the definition adjusted by the Centers for Disease Control and Prevention's, since the previous definition of the World Health Organization mentioned persistent symptoms after 12 wk[1].

However, for all these etiopathogenic processes that define long COVID to occur, there is still no specific evidence on it and various theories have been created that try to decipher the pathophysiology of said entity, some are independent, but others have strong ties between them. And depend on each other, the main etiopathogenic theories of long COVID are mentioned below:

Theory of viral persistence or viral particles: There is evidence that after an acute episode of COVID-19, there is persistence of viral particles in various organs up to one year after the episode and the organs mainly affected are: Brain, Gastrointestinal and Hemolymphatic; Similarly, they have been detected in blood, feces and urine[2,3].

Theory of endothelial dysfunction: This theory deals with the damage to the vascular endothelium that leads to endothelitis which, in turn, will favor platelet increase and activation, increased risk of thrombus formation with subsequent damage to organs and tissues by a mechanism of tissue ischemia that can affect the main organs and systems of the human body[4,5].

Theory of platelet hyperactivity: Which is related to the previous theory and mentions that this hyperactivity favors the formation of microthrombi which have the characteristic of being amyloid and in this area, is linked to the theory of viral persistence which mentions that protein S has amyloidogenesis potential, which makes these amyloid thrombi more resistant to degradation and more hard, which ensures occlusion of the microvasculature with subsequent diverse organic damage (highlights tissue destruction and damage to central and peripheral nerves)[6-8].

Crucial nerve damage theory: This theory ties in with the previous one and refers to damage to nerves crucial to the functioning of the autonomic nervous system, such as damage to the vagus nerve, which controls various functions of the cardiovascular systems, gastrointestinal and pulmonary, so damage to it can cause many symptoms in these systems[9].

Theory of immune abnormalities: After an acute COVID-19 case, there is evidence of persistent inflammation that feeds the inflammasome of each person, in addition to the presence of autoimmunity that adds various comorbidities to the patients and even the *de novo* appearance of rheumatological diseases such as lupus, dermatomyositis, rheumatoid arthritis, *etc.* In addition, the production of antibodies against the angiotensin converting enzyme-2 (ACE2) receptor has been demonstrated that could decrease the activity of ACE2, both in the soluble part and in the membrane-bound part, which would finally activate the immune system, which can act as immunological priming by molecular mimicry. In the same way, an "exhaustion of the immune system after an acute COVID-19 condition has been demonstrated, which conditions a decrease in lymphocyte subpopulations and the subsequent risk of opportunistic diseases[10-14].

Theory of interaction with subclinical viruses: This theory mentions that after the maladjustment of the immune system produced by acute COVID-19, some viruses that tend to remain in a subclinical form (mainly those of the herpesviridae family), can be activated again by adding morbidity to the long COVID picture, with various symptoms according to the viral type[15-17].

Dysbiosis theory: This theory mentions that patients with Long COVID present a dysbiosis which would hinder the relationships between the microbiota and the virome, favoring symptoms of the main organ systems and systems, highlighting the involvement of the respiratory system and the gastrointestinal system (Which have a large number of ACE2 and transmembrane serine protease 2 receptors that favor viral entry into cells), which are the main ones that harbor the microbiota, conditioning dysbiosis[18,19].

Theory of aggravation of chronic diseases or de novo appearance of chronic diseases: in this etiopathogenic theory it is mentioned that diseases previously diagnosed with an acute picture of COVID-19 can get out of control or worsen concomitantly, which adds greater comorbidity to the patient both in the acute stage as in long COVID; It has also been seen that after the acute picture, many patients develop chronic degenerative diseases such as: Diabetes, Hypertension, various Cardiopathies, Dementias, Thyroid diseases, *etc*[20-26].

Once the main theories have been described, we will try to put them together in order to be able to describe pathophysiology.

In recent times, viral persistence has stood out, mainly of the S and N proteins that greatly affect the central and autonomic nervous system, coupled with the neurotropism of SARS-CoV-2 that causes damage to the nervous system and the vagus nerve, hence the main manifestations are neuropsychiatric; protein S has the particularity of conditioning amyloidogenesis together with the presence of amyloid peptide A product of inflammation in the acute stage, which could lead to the presence of amyloid microthrombi, perpetuating both inflammation and thrombotic risk; in aggregate form, these characteristics can condition both positive and negative immune deregulation; positive deregulation would increase the activity of the immune system conditioning autoimmunity while negative deregulation is associated with completeness of the system with disorder of B, T and NK cell lines, the latter reveals the reactivation of latent viruses that increase morbidity as well as the virome, causing an imbalance between it and the intestinal microbiota, giving way to the theory of dysbiosis, which is associated, in addition to gastrointestinal symptoms, to neuropsychiatric and cardiovascular disorders, even leading to dysautonomia and hormonal changes, leading to a vicious circle.

Table 1 Diagnosis and treatment based on the theories of long coronavirus disease

Pathological condition to study	Clinic	Basic diagnostic method	Extension studies	Treatments
Neuropsychiatric manifestations	Anxiety depression headache; brain fog; early dementia fatigue/weakness/myasthenia mitochondriopathy suspected	Psychological tests; clinical questioning neurological examination	Cranial CT head MRI brain PET scan CSF analysis; electroencephalogram EMG/CNV; serum lactate-pyruvate/CSF	Psychological therapy psychiatric treatment pacing; brain electrostimulation; speech therapy; behavioral therapy
Viral persistence	Leukopenia, lymphopenia virus reactivation (herpes, EBV); persistently positive COVID tests	Nasal COVID antigen (or PCR-RT); IgM-IgG serology for herpes, CMV, EBV	Total body PET to viral reservoirs, RT-PCR for Sars Cov2: Serum; urinary; stool	Antivirals: Paxlovid (Yale trial); oral remdesivir; acyclovir, ganciclovir
Immunothrombosis	Clinical data of inflammation or thrombosis: Arthralgias/arthritis; myalgias; arterial/venous thrombosis	D-dimer ferritin; C reactive protein reactive thrombocytosis DHL; creatine phosphokinase myoglobin	Intentional search for amyloid microthrombi: Immunofluorescence microscopy; flow cytometry; alpha 2 antiplasmin; serum amyloid A platelet hyperactivation; platelet aggregometry	Triple therapy: Oral anticoagulants; dual antiplatelet; gastric protection. Fibrinolytics chelators vitamins
Immune dysregulation	Frequent infections; de novo appearance of autoimmune diseases	Leukopenia, lymphopenia reactive lymphocytosis	Lymphocyte subpopulation; CD4/CD8/natural killers; miscellaneous and specific antibodies	Immunomodulators immunostimulants biological therapy; monoclonal antibodies (temelimab)
Vagus nerve injury	Brain fog dysautonomias	Electrocardiogram holter; ambulatory blood pressure monitoring	Vagus nerve ultrasound tilted table test	
Dysbiosis	Brain fog depression/anxiety irritable colon chronic diarrhea	Coprological stool culture	Intestinal dysbiosis test stool calprotectin dysbiosis specific kits: Gastrotest; GI effects; healthy gut	
Miscellany	Hepatic steatosis chronic kidney failure dysthyroidisms; chronic lung disease		Kidney, liver, thyroid function female hormonal profile spirometry, chest X-ray, chest CT	Specific treatments
Commercial kits for persistent COVID diagnosis			CheqUp; IncellKINE	

CT: Computed tomography; CMV: Cytomegalovirus; EMG: Electromyography; EBV: Epstein-Barr virus; GI: Gastrointestinal; MRI: Magnetic resonance imaging; PET: Positron emission tomography; PCR-RT: polymerase chain reaction reverse transcriptase; IgM: Immunoglobulin M; IgG: Immunoglobulin G; COVID: Coronavirus disease.

There are currently many researcher-led treatment efforts and initiatives, notably the following:

Dr. Iwasaki from Yale University, leads a clinical study using Paxlovid in these patients affected by Long COVID, based on the theory of viral persistence, who receive the antiviral for 15 days waiting for symptomatic improvement, the study is still recruiting participants and promising results are expected (ClinicalTrials.gov Identifier: NCT05668091)[27].

Other researchers led by Dr. Etheresia Pretorius, are addressing the theory of immunothrombosis, in which they have demonstrated the presence of amyloid microthrombi and initiated a special therapy called Triple therapy, which uses direct oral anticoagulants, dual antiplatelet therapy, and gastric protection, from which encouraging results are expected[28,29].

Other research efforts fall on two researchers, Tamara Romanuk and Ale Frost, who address the theory of dysbiosis secondary to long COVID and that it conditions a gut-brain axis disorder with subsequent neuropsychiatric dysfunction (@remissionbiome on twitter); In their study, they have implemented the study of the microbiota and are using a treatment scheme with the antibiotics doxycycline and amoxicillin with clavulanate, taking advantage of properties against neuroinflammation and neuroimmunology; something similar occurs with the study of dectin-1 as a therapeutic target for the treatment of stress-induced behaviors[30,31].

In Spain, researchers address the theory of damage to the vagus nerve in patients with long COVID, based on the prevalence of inappropriate sinus tachycardia in these patients, they have devised an ultrasound protocol to identify vagus nerve disorders, which would corroborate the viral neurotropism that affects to said nerve, conditioning dysautonomia and disorders in other spheres such as neuropsychiatric, cardiopulmonary, gastrointestinal and endocrinological (Table 1)[32].

Finally, in Switzerland, a research protocol has begun with a monoclonal antibody called Temelimab focused on chronic fatigue and cognitive alterations, whose target is a protein called HERV-W-End, which has been associated with autoimmune diseases and chronic fatigue; encouraging results are expected for long COVID patients. The investigation has been extended to Spain and Italy (ClinicalTrials.gov Identifier: NCT05497089)[33].

CONCLUSION

There is still much to be deciphered in the etiopathogenesis and pathophysiology of long COVID, however current efforts clarify these conditions on which treatments are tested in the real world, in order to limit the pathological manifestations that affect the population affected by long COVID. With the study of etiopathogenic theories, diagnostic and treatment strategies can begin to be created.

FOOTNOTES

Author contributions: Del Carpio-Orantes L contributed to conceptualized the idea of the review, searched for information for the review, wrote the document, provided a summary of the information on the diagnosis and treatment chart.

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ORIGINAL ARTICLE

Basic Study

Re-analysis of hepatitis B virus integration sites reveals potential new loci associated with oncogenesis in hepatocellular carcinoma

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Abstract

BACKGROUND

Hepatitis B virus (HBV) is a major cause of hepatocellular carcinoma (HCC). HBV DNA can get integrated into the hepatocyte genome to promote carcinogenesis. However, the precise mechanism by which the integrated HBV genome promotes HCC has not been elucidated.

AIM

To analyze the features of HBV integration in HCC using a new reference database and integration detection method.

METHODS

Published data, consisting of 426 Liver tumor samples and 426 paired adjacent non-tumor samples, were re-analyzed to identify the integration sites. Genome Reference Consortium Human Build 38 (GRCh38) and Telomere-to-Telomere Consortium CHM13 (T2T-CHM13 (v2.0)) were used as the human reference genomes. In contrast, human genome 19 (hg19) was used in the original study. In addition, GRIDSS VIRUSBreakend was used to detect HBV integration sites, whereas high-throughput viral integration detection (HIVID) was applied in the original study (HIVID-hg19).

RESULTS

A total of 5361 integration sites were detected using T2T-CHM13. In the tumor samples, integration hotspots in the cancer driver genes, such as *TERT* and *KMT2B*, were consistent with those in the original study. GRIDSS VIRUSBreakend detected integrations in more samples than by HIVID-hg19. Enrichment of integration was observed at chromosome 11q13.3, including the *CCND1* pro-

moter, in tumor samples. Recurrent integration sites were observed in mitochondrial genes.

CONCLUSION
GRIDSS VIRUSBreakend using T2T-CHM13 is accurate and sensitive in detecting HBV integration. Re-analysis provides new insights into the regions of HBV integration and their potential roles in HCC development.

Key Words: Carcinoma; Hepatocellular; Hepatitis B virus; Virus integration

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Core Tip: To understand the role of hepatitis B virus (HBV) in hepatocellular carcinoma (HCC) development, we re-analyzed HBV integration sites using publicly available data. We found that chromosome 11q13.3 is a frequently observed HBV integration site. This region contains important cancer driver genes, such as *CCND1* and *FGF19*, which are amplified in HCC. This finding supports a mechanism of carcinogenesis promoted by HBV-induced genomic instability in the liver and provides insights into treating a subset of liver cancers.

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INTRODUCTION

The hepatitis B virus (HBV) is a major cause of hepatocellular carcinoma (HCC). When HBV infects liver cells, HBV DNA can be integrated into the human genome. Integration events typically occur during the early stages of an infection[1,2], and are known to promote carcinogenesis *via* several mechanisms: (1) Increasing the expression levels of neighboring genes; (2) induction of genomic instability and somatic copy number alterations of genes; (3) deletion of tumor suppressor genes through structural mutations [3]; and (4) inducing expression of HBV X protein (HBx) or HBx fusion proteins that contribute to carcinogenesis.

To investigate the effect of HBV on hepatocarcinogenesis, several studies have been conducted using next-generation sequencing technology to identify integration sites of HBV DNA. Examples of such technologies include whole genome sequencing[4] and HBV capture sequencing[5]. These studies revealed frequent integration into the promoter regions of *TERT* and *KMT2B* in tumor tissues and *FN1* in normal tissues. In an examination of an HBV-infected human-hepatocyte chimeric mouse model, mitochondrial DNA (mtDNA) was thought to be a frequent site of integration[1]. A European study reported a lower frequency of *KMT2B* insertion and a higher frequency of integration into *ADH* genes in normal tissues[6].

Most previous studies have used Genome Reference Consortium Human Build 37 (GRCh37) or human genome 19 (hg19) as the reference genomes. In GRCh37/hg19 and Genome Reference Consortium Human Build 38 (GRCh38)[7], tandem repeats, microsatellites, and minisatellites found in telomeres and centromeres remained unresolved. The complete human genome sequence, Telomere-to-Telomere Consortium CHM13 (T2T-CHM13 (v2.0))[8], was released in 2022.

Various methods have been used to detect integration breakpoints. High-throughput viral integration detection (HIVID), a detection method based on a pair-read assembly strategy[9], was applied in the analysis of 426 HCC cases[5]. GRIDSS is a multithreaded structural variant caller from a combination of assembly, split read, and read pair support[10]. VIRUSBreakend utilizes a virus-centric variant calling and assembly approach to identify viral integrations with high sensitivity and low false discovery rate, allowing the identification of integrations in repetitive host regions[11].

Here, we report new features observed by re-analyzing the published data using GRIDSS VIRUSBreakend based on GRCh38 and T2T-CHM13.

MATERIALS AND METHODS

Sequence data were obtained from the Sequence Read Archive (SRA) with accession number SRA335342

[5]. The dataset consisted of 426 tumor samples and 426 paired adjacent non-tumor samples.

All reads in the dataset were aligned to the GRCh38 and T2T-CHM13 reference genomes using bwa-mem2[12,13]. VIRUSBreakend was used to detect integration sites (Supplementary Figure 1), and the analysis was performed using Nextflow[14] on Amazon Web Service. HBV integration sites were detected using GRIDSS VIRUSBreakend[11]. Integration sites were compared with the count of fragments providing breakend for the variant allele (BVF) in the variant call format files. Statistical analysis and visualization were performed using R software, and statistical significance was set at $P < 0.05$.

RESULTS

Comparison of HBV integration sites

In total, 5361 and 5198 integration breakpoints were detected with T2T-CHM13 and GRCh38, respectively. The breakpoints were similar between the references using GRCh38 and T2T-CHM13 (Figure 1A and B). Consistent with previous studies, integration breakpoints were enriched in the *TERT* promoter region in tumor samples. In contrast, integration into *FN1* was frequently observed in non-tumor samples.

Compared with the original study, our analysis detected integrations in more samples (357 *vs* 328 in tumors; 288 *vs* 160 in non-tumors) (Table 1). In addition, we detected integration in the *TERT* region in 105 tumor samples, whereas the original study observed integration in 95 tumor samples (Table 2). In contrast, the number of breakpoints detected in tumors was lower than that in the original study (Table 1). In our study, only breakpoints validated by VIRUSBreakend were counted (Supplementary Figure 2). Integration of *DDX11L* was frequently detected in the original study, but no integration breakpoints were detected in our study (Table 2). The *DDX11L* gene family is frequently detected as a target for integration using a capture sequencing approach, but it is possible that fragments were mapped incorrectly owing to repetitive sequences[15,16]. In the non-tumor samples, our study detected more integrations, both in the number of samples and breakpoints. For example, we detected 97 integration breakpoints in the *FN1* gene from 56 non-tumor samples. The earlier analysis detected only 19 breakpoints from 17 non-tumor samples (Table 2). Few oncogenic regions were affected in the non-tumor samples. Breakpoints were most frequent around direct repeat 1 of the HBV genome (Figure 1C).

Chromosome 11q13.3 is a frequent site for HBV integration

When the chromosome region was explored, we found that the integration breakpoint at 11q13.3 was enriched with T2T-CHM13 and GRCh38 (Figures 2A-C). Breakpoints at 11q13.3 were more frequent in the tumor samples than in the non-tumor samples (16 (3.8%) of tumor samples compared to 1 (0.02%) of non-tumor samples, Figure 2B). 11q13.3 is characterized by the evolutionarily well-conserved genes *CCND1*, *FGF19*, *FGF4*, and *FGF3*[17], where copy number amplification frequently occurs in tumors (Figure 2D)[18,19]. Some breakpoints were within the genic and promoter regions of the genes, including *CCND1* and *FGF4*. Integration appeared to be distributed more in the non-genic regions (Figure 2B). When fragments from the integration site were counted using BVF, the values were higher in tumor samples than in non-tumor samples (Figure 2E). High BVF value formed a peak in the 11q13.3 in addition to the peak in the *TERT*, *KMT2B*, and *CCNE1* genes in the tumor samples (Figure 2E and Supplementary Figure 3).

Mitochondrial DNA has sites where HBV DNA is frequently integrated

There is some debate regarding whether mtDNA is a frequent site of HBV integration. A study using a mouse model by Furuta *et al*[1] found that mtDNA was frequently integrated early in infection. More recently, a preprint suggested that mtDNA is indeed a site for integration[20,21]. Although the original paper on which this study was based did not mention integration into mitochondria, we detected many integration breakpoints into mtDNA and identified repeat integration sites (Table 3 and Figure 3)[22]. Integration breakpoints in mtDNA were observed in both tumor and non-tumor samples. Recurrent integration events were observed in *ND4*. Of these, eight events were from non-tumor samples, and two from tumor samples. Microhomologous sequences were observed in some regions. For example, the GCCNTTCTCATC sequence, where N represents any nucleotide or gap, was observed at the junction of the *ND4* gene (Chromosome M:11079) and the HBV genome (HBV:1559). In contrast, the GCTTCACC sequence was observed at the junction of the *ND4* gene (Chromosome M:11104) and the HBV genome (HBV:1590). It is also possible that these integration breakpoints exist in nuclear-mitochondrial segments.

Table 1 Comparison of hepatitis B virus integration breakpoints among reference genomes

	GRCh38	T2T-CHM13	Original
Tumor			
Number of breakpoints	2439	2487	3486
Number of samples	357	355	328
Non-tumor			
Number of breakpoints	2759	2874	739
Number of samples	288	288	160

Table 2 Comparison of frequent integration breakpoints in the samples

Gene	GRCh38		Original	
	Breakpoints (*n*)	Samples (*n*)	Breakpoints (*n*)	Samples (*n*)
Tumor				
TERT	150	105	160	95
KMT2B	56	33	55	30
DDX11L1	0	0	36	23
CCNA2	12	7	14	8
CCNE1	13	9	14	7
Non-tumor				
FN1	97	56	19	17
TERT	12	10	8	3
IQGAP2	7	5	1	1
KMT2B	7	4	5	3

DISCUSSION

In this study, GRIDSS VIRUSBreakend, with an updated human reference genome, was used to detect HBV integration using public sequencing data from liver tumor and non-tumor samples. HBV integration was detected in more samples than in the original analysis (Table 1). The difference in methods could account for the discordant results. We investigated an example of HBV integration sites in the *TERT* region detected by GRIDSS VIRUSBreakend, but not in the original study (Supplementary Figures 4 and 5). In the original study, the HIVID pipeline, based on paired-end read assembly, was applied to detect integration[9]. In the sequencing data, some paired-end reads could not be assembled because of the absence of overlapping bases. These reads were also included in our analysis to detect integration sites more accurately. It should be noted that the GRIDSS VIRUSBreakend uses genotype D HBV for viral genome reference, whereas genotype C HBV is dominant in the current dataset, which may affect the sensitivity of virus detection.

We found HBV integration clusters in the 11q13.3 region (Figure 2). Unlike previously known single gene integration sites, such as *TERT* and *KMT2B*, 11q13.3 spans multiple gene regions. Although these clusters can be observed in the supplemental data of the original paper, to our knowledge, it has not been previously mentioned. Enrichment of 11q13.3 was more significant in tumors than in non-tumor tissues. *CCND1*, *FGF19*, *FGF4*, and *FGF3* are located at 11q13.3, where copy number amplification frequently occurs in tumors.

Integration into *CCND1*, located at 11q13.3, is a potential driver event[23], but its frequency is not high. Although recent studies have not detected integration at 11q13.3[1,6], several studies have detected these events only as supplementary data[4,5,24] and they have been reported since 1988[25,26]. According to a study by Bok *et al*[27], the expression levels of cancer-related genes, including *CCND1* and *FGF19*, are elevated near the viral integration site on 11q13.3 in an HCC cell line. HBV integration at this locus may be linked to cancer gene activation, as *FGF19* amplification was associated with chronic HBV infection[28,29].

HBV integration may be associated with copy number alterations[3]. Chromosomal instability often leads to copy number alterations in the short and long arms of the chromosome. However, 11q13.3

Table 3 Hepatitis B virus integration breakpoints in mitochondrial DNA

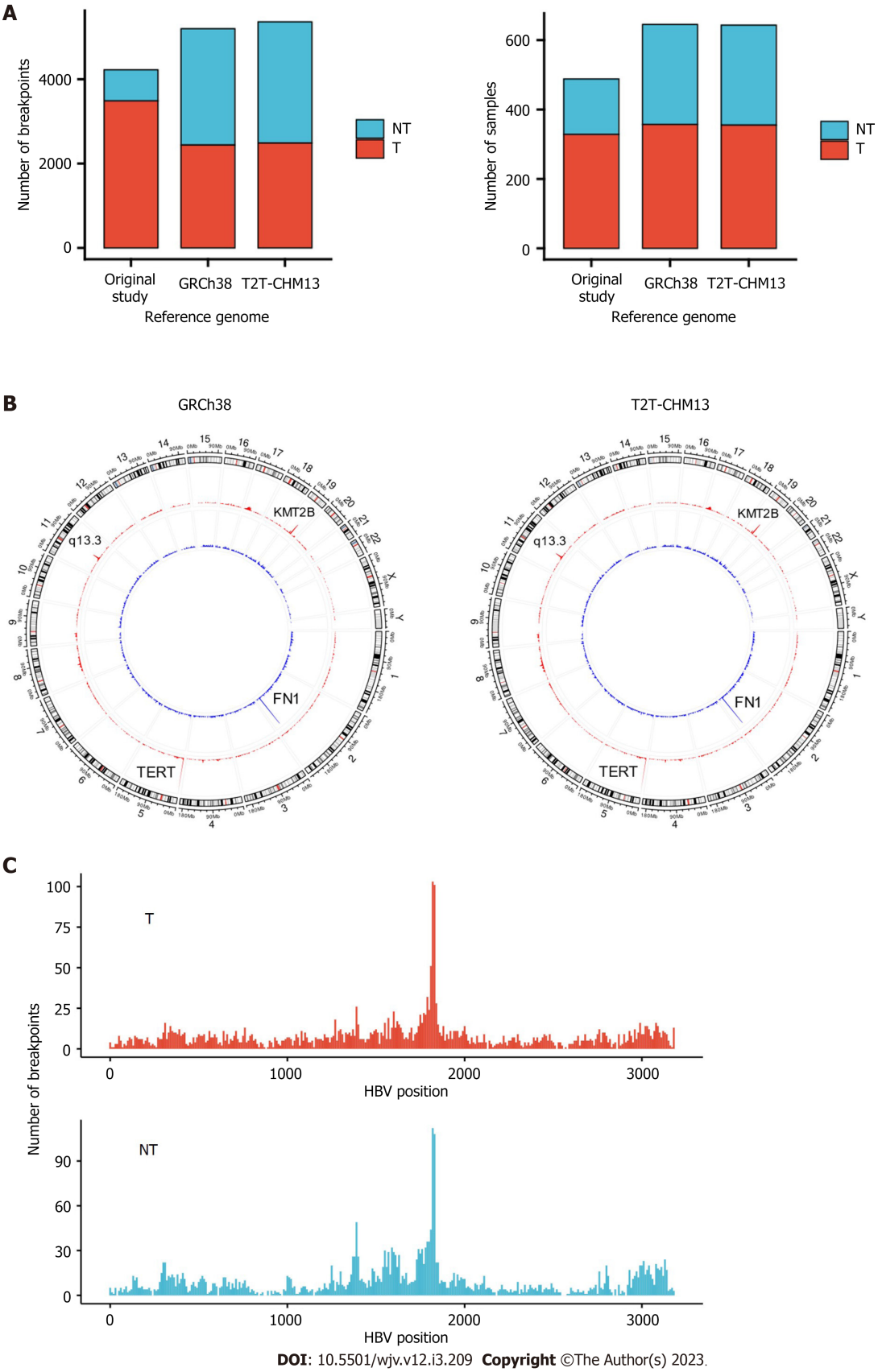
Sample	Tumor/Non-tumor	Chromosome	Position	HBV	Quality score
SRR3104746	NT	chrM	5367	1247	6933.33
SRR3105143	NT	chrM	5682	1513	11817.58
SRR3105012	T	chrM	8220	112	2080.04
SRR3104491	NT	chrM	8524	2482	33322.16
SRR3105095	NT	chrM	8694	471	4209.06
SRR3105101	NT	chrM	11079	1559	2937.33
SRR3105143	NT	chrM	11079	1559	14876.83
SRR3105001	NT	chrM	11104	1590	45538.21
SRR3105149	NT	chrM	11104	1590	16156.63
SRR3105251	NT	chrM	11104	1590	2276.31
SRR3105293	NT	chrM	11104	1590	24562.69
SRR3104643	T	chrM	11104	1590	13683.16
SRR3105172	T	chrM	11126	1621	2610.44
SRR3105251	NT	chrM	11130	1625	6793.43
SRR3104939	NT	chrM	11139	1729	30830.54
SRR3105149	NT	chrM	12453	323	2345.03
SRR3104636	NT	chrM	12735	1381	26840.64
SRR3105083	NT	chrM	13273	1755	4230.85
SRR3104696	NT	chrM	13433	363	2963.36
SRR3104643	T	chrM	13964	2809	2222.86
SRR3104823	NT	chrM	14052	1017	1788.18
SRR3105049	NT	chrM	14892	1768	21102.87
SRR3104982	T	chrM	15679	1768	32371.78
SRR3105185	NT	chrM	16319	1788	2013.53

HBV: Hepatitis B virus.

causes strong copy number amplification in a localized region in the middle of the chromosome (Figure 2D). Previous results using whole genome sequencing indicated that the integration allele frequency was high in the tumor samples, especially in the recurrent integration in tumors such as *TERT* [4]. By comparing fragment counts from the integration site using BVF, the values were found to be higher in the tumor samples than in the non-tumor samples. Some of the integration breakpoints at 11q13.3 had extremely high fragment counts (Figure 2E and Supplementary Figure 3). If BVF correlates with the integration allele frequency, it is possible that these events reflect the clonal expansion of tumors with integration breakpoints or the amplification of integrated genes. *CCND1-FGF19* amplification occurred at later points in the evolution of HCC[30]. Further research is needed to investigate the relationships between integration, copy number alteration, and cancer gene activation at 11q13.3.

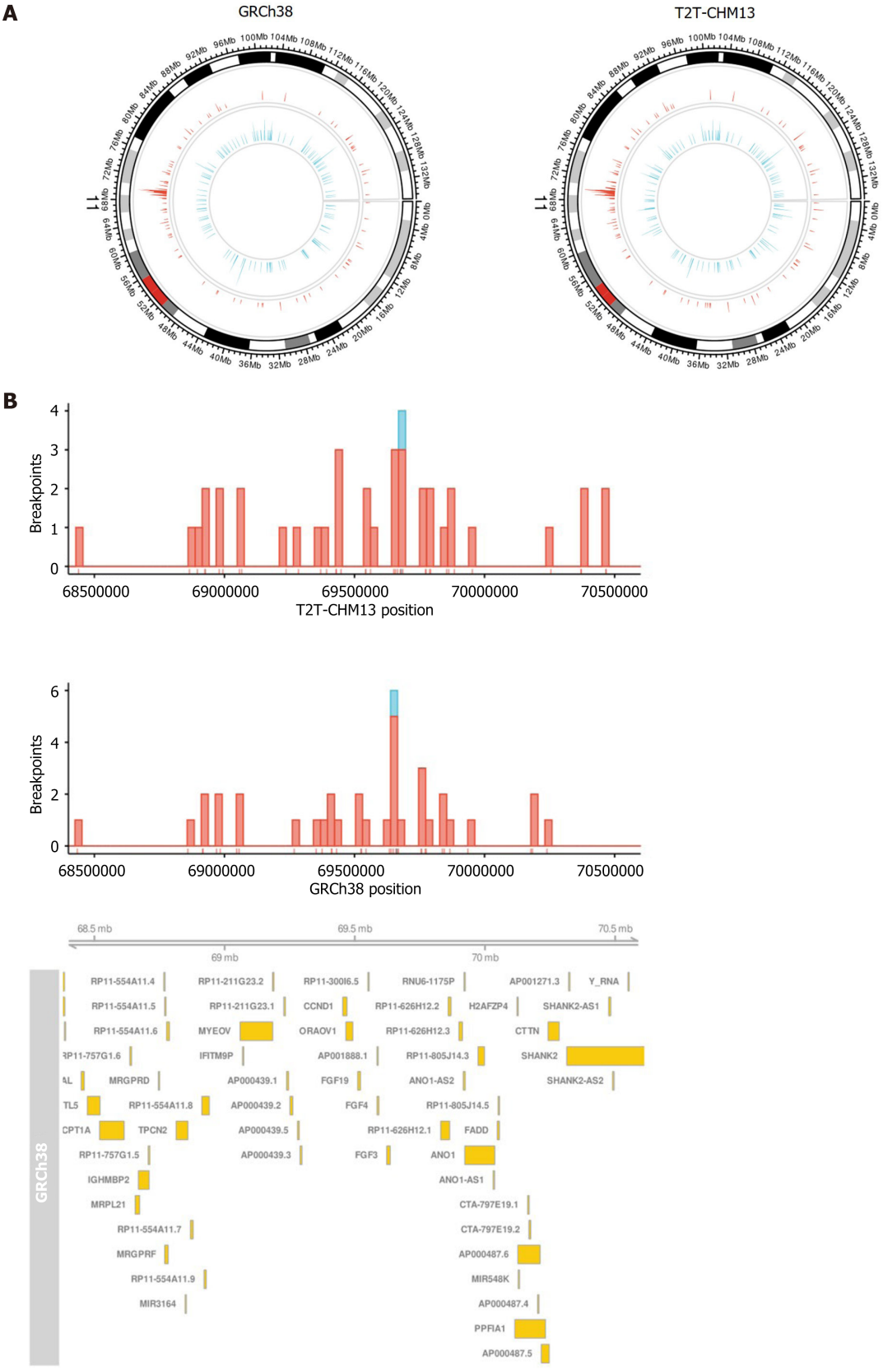
In our analysis, HBV integration in the mtDNA was observed in 2.3% (20/852) of the samples, and the *ND4* gene was a frequent target of HBV integration (Table 3). According to a previous study, HBV integration into mtDNA has occurred in only 0.1% of human clinical liver tissues[1]. Mouse model experiments have suggested that this integration primarily occurs during the early stages of HBV infection through microhomology-mediated end joining[1]. It is also possible that HBV integration occurs in nuclear copies of mtDNA sequences rather than in the mitochondria. Giosa *et al*[21] detected HBV integration in DNA isolated from mitochondria. The D-loop region is the target of HBV integration. Our analysis suggests that *ND4* genes may also be targeted for integration through microhomology-mediated mechanisms.

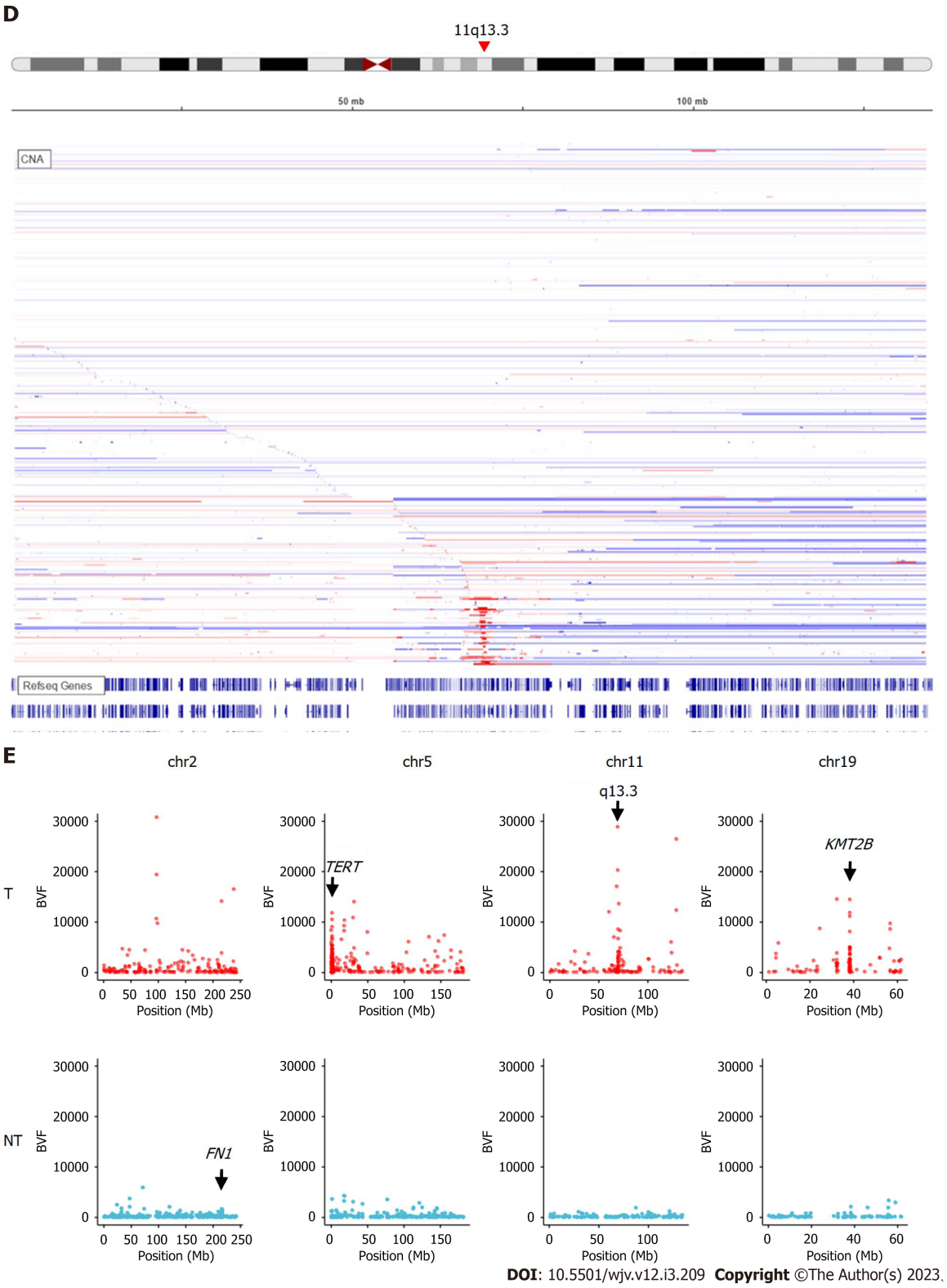
This study has several limitations. First, the analysis was conducted using existing data and the findings were not validated using independent data. Second, the original data were based on HBV



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Figure 1 Hepatitis B virus integration breakpoints across the reference genomes. A: Integration breakpoints in the human reference genomes in tumor and non-tumor samples; B: Circos plot of integration breakpoints. Red represents tumor samples, and blue represents non-tumor samples; C: Hepatitis B virus genome integration breakpoints. T: Tumor; N: Non-tumor.

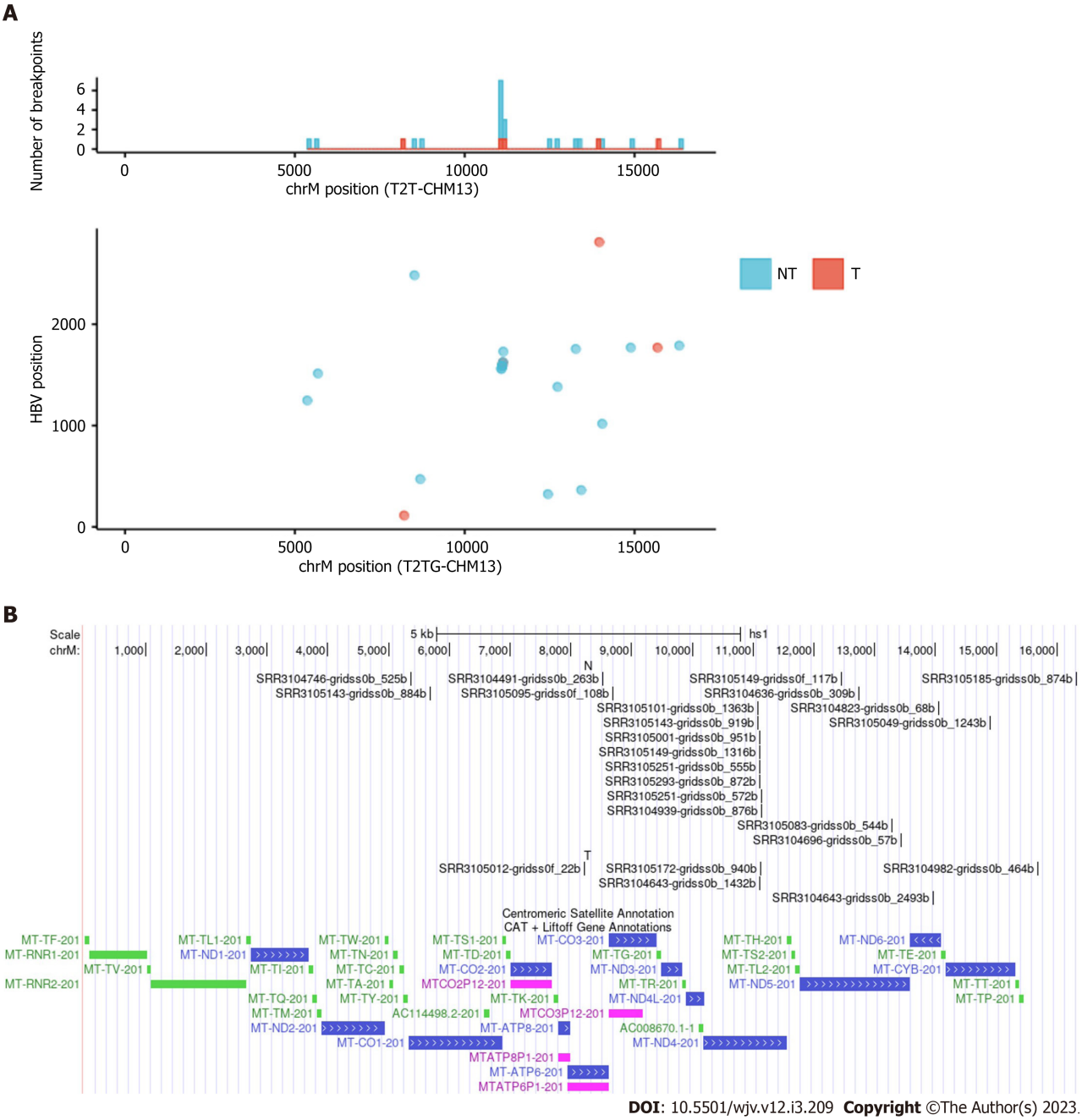

A
GRCh38
T2T-CHM13
B
Breakpoints
T2T-CHM13 position
GRCh38 position
68.5 mb
69.5 mb
70.5 mb
69 mb
70 mb
GRCh38
C
Number of breakpoints
3.07e-35
1.21e-38
Actual
Expected
GRCh38 11q13.3
T2T-CHM13 11q13.3



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Figure 2 Integration breakpoints at chromosome 11. A: Circos plot of breakpoints at chromosome 11 in the human reference genomes; B: Integration breakpoints around 11q13.3 in relation to coding genes retrieved from Ensembl. Red represents tumor samples, and blue represents non-tumor samples; C: Comparison of integration breakpoints around 11q13.3 in the tumor samples. Actual represents actual number of integration breakpoints. Expected represents expected number of integration breakpoints assuming random distribution; D: Copy number of liver cancer samples from cBioPortal[18,19]. Red represents amplification, and blue represents deletion. E: Distribution of the number of fragments that provide breakend for the variant allele. T: Tumor; NT: Non-tumor.

capture sequencing, and gene copy numbers were not available. Finally, the integration data were obtained from short-read sequencing and have not been validated using long-read sequencing data.



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Figure 3 Integration breakpoints in the mitochondrial genome. A: The upper panel displays integration breakpoints across mitochondrial genomes according to tumor and non-tumor samples, and the lower panel shows integration breakpoints along the human and hepatitis B virus genomes. Red represents tumor samples, and blue represents non-tumor samples; B: Integration breakpoints on the mitochondrial genome annotated using UCSC genome browser (NT: Non-tumor; T: Tumor)[22].

CONCLUSION

HBV integration in HCC samples has been characterized using the complete human reference. GRIDSS VIRUSBreakend using T2T-CHM13 is accurate and sensitive in detecting HBV integration. HBV frequently integrates at the 11q13.3 region, where the *CCND1* gene is located, and this region is frequently amplified in several types of cancer, including HCC. Further research is needed to examine how HBV integration interacts with driver gene expression and copy number alteration.

ARTICLE HIGHLIGHTS

Research background

Many hepatitis B virus (HBV)-infected patients suffer from hepatocellular carcinoma (HCC), but a little focus is given to detect HBV integration pattern in the treatment of HCC. Detection of HBV integration can be improved by introducing a reliable detection method.

Research motivation

HBV frequently integrates at the 11q13.3 region, where the *CCND1* gene is located, and this region is frequently amplified in several types of cancer, including HCC.

Research objectives

We aimed to analyze the features of HBV integration in HCC using a new reference database and integration detection method.

Research methods

Published data, consisting of 426 liver tumor samples and 426 paired adjacent non-tumor samples, were re-analyzed to identify the integration sites. Updated human reference genomes, Genome Reference Consortium Human Build 38 (GRCh38), and Telomere-to-Telomere Consortium CHM13 (T2T-CHM13 (v2.0)) were used. In addition, GRIDSS VIRUSBreakend, which utilizes a virus-centric variant calling and assembly approach, was used to detect HBV integration sites.

Research results

A total of 5361 integration sites were detected using T2T-CHM13. In the tumor samples, integration hotspots in the cancer driver genes, such as *TERT* and *KMT2B*, were consistent with those in the original study. GRIDSS VIRUSBreakend detected integrations in more samples than original analysis. Enrichment of integration was observed at chromosome 11q13.3, including the *CCND1* promoter, in tumor samples. Recurrent integration sites were observed in mitochondrial genes.

Research conclusions

GRIDSS VIRUSBreakend using T2T-CHM13 is accurate and sensitive in detecting HBV integration and provides new insights into the regions of HBV integration and their potential roles in HCC development.

Research perspectives

Further research is needed to examine how HBV integration interacts with driver gene expression and copy number alteration.

FOOTNOTES

Author contributions: Kojima R and Nakamoto S contributed to the conception, design, and writing of the manuscript; Kojima R contributed to data management and analysis; Kogure T, Ma Y, Ogawa K, Iwanaga T, Qiang N, Ao J, Nakagawa R, Muroyama R, Nakamura M, Chiba T, Kato J, and Kato N contributed to manuscript review and editing; Kato N contributed to the project administration.

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Conflict-of-interest statement: All the authors have no conflict of interest related to the manuscript.

Data sharing statement: All the data supporting this study are stored in the SRA database with accession number SRA335342.

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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Retrospective Cohort Study

Association between alcohol-associated cirrhosis and inpatient complications among COVID-19 patients: A propensity-matched analysis from the United States

Faisal Inayat, Hassam Ali, Pratik Patel, Rubaid Dhillon, Arslan Afzal, Attiq Ur Rehman, Muhammad Sohaib Afzal, Laraib Zulfiqar, Gul Nawaz, Muhammad Hassan Naeem Goraya, Subanandhini Subramanium, Saurabh Agrawal, Sanjaya K Satapathy

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Abstract

BACKGROUND

Alcohol-associated cirrhosis (AC) contributes to significant liver-related mortality

in the United States. It is known to cause immune dysfunction and coagulation abnormalities. Patients with comorbid conditions like AC are at risk of worse clinical outcomes from coronavirus disease 2019 (COVID-19). The specific association between AC and COVID-19 mortality remains inconclusive, given the lack of robust clinical evi-dence from prior studies.

AIM

To study the predictors of mortality and the outcomes of AC in patients hospitalized with COVID-19 in the United States.

METHODS

We conducted a retrospective cohort study using the National Inpatient Sample (NIS) database 2020. Patients were identified with primary COVID-19 hospitalizations based on an underlying diagnosis of AC. A matched comparison cohort of COVID-19 patients without AC was identified after 1:N propensity score matching based on baseline sociodemographic characteristics and Elixhauser comorbidities. Primary outcomes included median length of stay, median inpatient charges, and in-hospital mortality. Secondary outcomes included a prevalence of systemic complications.

RESULTS

A total of 1325 COVID-19 patients with AC were matched to 1135 patients without AC. There was no difference in median length of stay and hospital charges in COVID-19 patients with AC compared to non-AC ($P > 0.05$). There was an increased prevalence of septic shock (5.7% *vs* 4.1%), ventricular fibrillation/ventricular flutter (0.4% *vs* 0%), atrial fibrillation (13.2% *vs* 8.8%), atrial flutter (8.7% *vs* 4.4%), first-degree atrioventricular nodal block (0.8% *vs* 0%), upper extremity venous thromboembolism (1.5% *vs* 0%), and variceal bleeding (3.8% *vs* 0%) in the AC cohort compared to the non-AC cohort ($P < 0.05$). There was no difference in inpatient mortality in COVID-19 patients with non-AC compared to AC, with an odds ratio of 0.97 (95% confidence interval: 0.78-1.22, $P = 0.85$). Predictors of mortality included advanced age, cardiac arrhythmias, coagulopathy, protein-calorie malnutrition, fluid and electrolyte disorders, septic shock, and upper extremity venous thromboembolism.

CONCLUSION

AC does not increase mortality in patients hospitalized with COVID-19. There is an increased association between inpatient complications among COVID-19 patients with AC compared to non-AC.

Key Words: Alcoholic cirrhosis; COVID-19; Chronic liver disease; Mortality predictors; Inpatient complications

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Core Tip: High-risk comorbid conditions significantly increase the mortality linked to coronavirus disease 2019 (COVID-19). In this large National Inpatient Sample-based retrospective cohort study, we aimed to evaluate the specific clinical impact of alcohol-associated cirrhosis (AC) on patients hospitalized with COVID-19. We analyzed the patient outcomes based on comorbidities, mechanical ventilation, intensive care unit admission, and mortality predictors. Our findings show that AC does not increase mortality in patients hospitalized with COVID-19. Pertinently, there is an increased association between inpatient complications and COVID-19 patients with AC compared to non-AC. Predictors of mortality included advanced age, cardiac arrhythmias, coagulopathy, protein-calorie malnutrition, fluid and electrolyte disorders, septic shock, and upper extremity venous thromboembolism.

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INTRODUCTION

The global pandemic of coronavirus disease 2019 (COVID-19) has profoundly affected patients with pre-existing comorbidities. The World Health Organization reported that there were 768 million confirmed cases and 6.9 million fatalities as of June 21, 2023[1]. The disease presents a broad range of clinical features, from asymptomatic respiratory infection to severe pneumonia, thromboembolism, and even death. Initial reports from Wuhan indicated elevated serum liver biochemistry panels and associated liver injury in COVID-19 patients[2]. Previous research has demonstrated that patients with chronic liver disease (CLD) had a longer disease duration and higher mortality after contracting COVID-19[3,4]. CLD is a sig-

nificant public health concern, with an estimated global burden of 1.5 billion patients and 2 million annual deaths[5]. It is notable that viral hepatitis remains the leading cause of cirrhosis worldwide[6]. However, obesity and alcohol use have emerged as critical risk factors following advances in the prevention and treatment of viral hepatitis[6]. This etiological shift is expected to shape the future epidemiology of CLD[6]. It can potentially make the hepatic involvement of COVID-19 particularly troubling for patients with underlying alcohol-associated cirrhosis (AC)[7].

Patients with cirrhosis often develop immunological perturbations, leading to systemic inflammation and immune deficiency[8]. This immune dysfunction could potentially make it more difficult for cirrhotics to fight COVID-19. Numerous studies have identified an increased risk of COVID-19 severity and death in cirrhotic patients[9-11]. However, the literature offers conflicting results regarding the clinical impact of underlying cirrhosis on COVID-19 outcomes. For instance, Bajaj *et al*[12] reported similar mortality rates between cirrhotics with and without COVID-19, yet highlighted higher mortality in cirrhotics compared to patients suffering from COVID-19 alone. Moreover, the COVID-19-associated mortality rates in patients with CLD and cirrhosis varied from 8.9% to 39.8%[13,14].

Based on available clinical epidemiologic studies, there is a limited understanding of the etiologic basis of the clinical outcomes of cirrhosis in patients with COVID-19. However, several studies have investigated this subject. For example, in a single-center retrospective analysis of a cohort of patients hospitalized for COVID-19 with a prevailing alcoholic CLD, liver cirrhosis was linked to a fourfold increase in 30-d mortality[15]. A Korean study suggested that people with underlying nonalcoholic fatty liver disease may be more likely to test positive for severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) and experience severe infection[16]. In addition, Kim *et al*[17] conducted a multicenter, observational cohort study demonstrating increased overall mortality in patients with CLD and COVID-19 due to alcohol-associated liver disease, decompensated cirrhosis, and hepatocellular carcinoma.

Our objective is to assess the impact of AC on outcomes and mortality in patients hospitalized with COVID-19 using a large, multicenter database. We also seek to identify mortality predictors in COVID-19 patients with AC. To our knowledge, this is the first study to examine the specific clinical influence of AC on COVID-19 by assessing substantial epidemiological trends in hospitalized patients in the United States.

MATERIALS AND METHODS

Design and data source

This retrospective cohort study is reported according to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines[18]. We utilized the recently released National Inpatient Sample (NIS) database 2020. It is designed by the Agency for Healthcare Research and Quality[19]. NIS is the largest inpatient database in the United States healthcare system[19]. The design of this database enables the calculation of national estimates using sampling weights and a 20% stratified sample of hospitals[19]. Detailed information on the design of NIS and sampling methods is available at https://www.hcup-us.ahrq.gov. NIS 2020 utilized the International Classification of Diseases (ICD) 10 coding system to store and report data. We identified hospitalizations with a primary diagnosis (DX1) of COVID-19 using the "U07.1" ICD-10 code, which was introduced in March 2020[20]. Hospitalizations were excluded if the patient age was < 18 years, individuals were transferred, and/or COVID-19 was listed as a secondary diagnosis. Furthermore, patients were excluded if there was any history of cirrhosis due to nonalcoholic and other causes (viral, autoimmune, or non-specified), hepatocellular carcinoma, malignant neoplasm, end-stage renal disease requiring dialysis, quadriplegia, lymphoma, renal transplant, or liver transplant, as these were deemed high-risk conditions that could confound our analysis.

Outcome measures

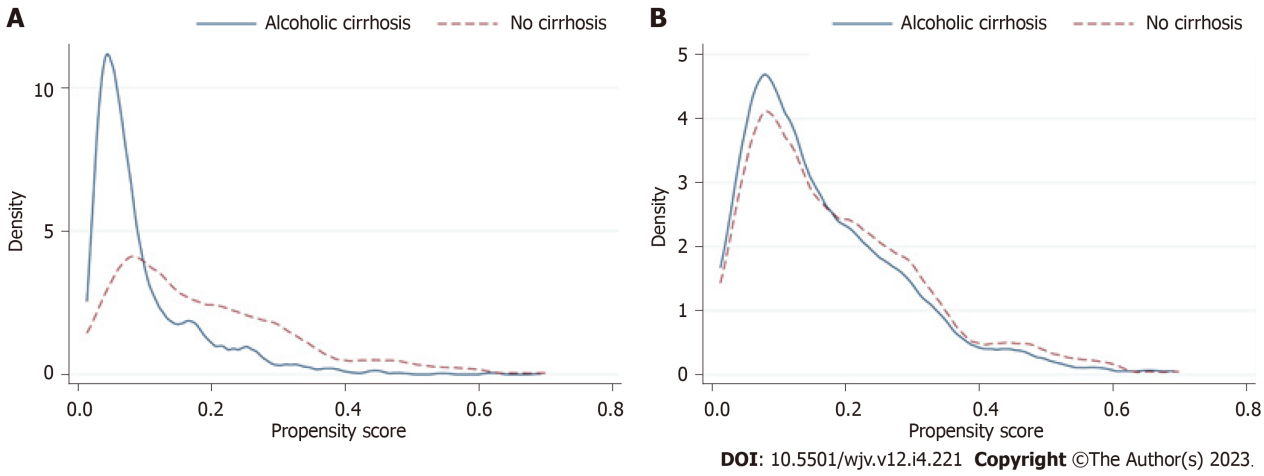
Primary outcomes included median length of stay, median inpatient charges, and in-hospital mortality. Secondary outcomes included a prevalence of respiratory, cardiac, circulatory, neurological, and renal complications.

Statistical analysis

Statistical analysis was performed using Statistical Software for Data Science (StataCorp LLC, College Station, TX, United States) version 16.0. Two cohorts were created based on the presence or absence of a secondary diagnosis of AC using ICD-10 codes employed in the published literature[21]. We developed matched cohorts using propensity score matching (PSM) to minimize the effect of comorbid imbalances between comparison cohorts. Each case was assigned a propensity score using a multivariable logistic regression that included baseline sociodemographic characteristics (age, sex, race, socioeconomic status, and Elixhauser comorbidities). Propensity scores between the two cohorts were matched in a 1:N fashion using the nearest-neighbor method within 0.01 standard deviations of the calculated score[22]. The covariate balance was then visualized using the two-way plot (Figure 1). A two-sample Wilcoxon rank-sum (Mann-Whitney) test was utilized for continuous variables. The Chi-square test was used to compare categorical variables. The significance threshold was set at $P < 0.05$. For logistic regression, hierarchical models were designed using any unbalanced variables in PSM (race). The positive mortality predictors were then used to build a final multivariate model. Only significant positive predictors of mortality were reported as odds ratios (OR) with 95% confidence intervals (CI) and P values.

Ethical considerations

The NIS is a de-identified hospital-level, third-party database. The privacy of patients, clinicians, and medical centers is protected by its design. Patient consent was waived as the hospitalization data were stripped of any patient identifiers. The approval of the institutional review board (IRB) was not required for this study.



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Figure 1 Two-way plot visualizing the covariate balance in our study. A: Before matching; B: After matching.

RESULTS

A total of 738010 primary COVID-19 hospitalizations fulfilled the selection criteria and were included in the study. A total of 1325 hospitalizations with AC were matched to 1135 without AC using nearest-neighbor matching (Table 1). The age group 50-64 years had a higher prevalence in the AC cohort than the non-AC cohort ($P < 0.001$). The median age was 60 years, with an interquartile range (IQR) of 54-67 in the AC cohort and 61 years (IQR: 54-67) in the non-AC cohort. There was no disparity based on gender ($P = 0.31$). Hispanics (33.2% *vs* 26.0%) and Native Americans (4.9% *vs* 4.8%) had a higher prevalence in the AC cohort compared to the non-AC cohort ($P < 0.001$). There was a higher prevalence of an Elixhauser Comorbidity Index (ECI) score ≥ 3 (98.9% *vs* 2.2%) in the AC group compared to the non-AC group ($P < 0.001$). Mortality was significantly higher in hospitalizations with non-AC compared to AC (15.0% *vs* 14.7%, $P = 0.024$). Disposition was more likely to be against medical advice if there was a secondary diagnosis of AC compared to non-AC (3.8% *vs* 3.5%, $P = 0.024$). There was no difference in intensive care unit (ICU)-level care (10.6% each, $P = 1.00$). There was no significant difference in median length of stay or median hospital charges between the AC and non-AC groups ($P > 0.05$).

There was an increased prevalence of septic shock (5.7% *vs* 4.1%), ventricular fibrillation/ventricular flutter (0.4% *vs* 0%), atrial fibrillation (13.2% *vs* 8.8%), atrial flutter (8.7% *vs* 4.4%), first-degree atrioventricular nodal block (0.8% *vs* 0%), upper extremity venous thromboembolism (VTE) (1.5% *vs* 0%), variceal bleeding (3.8% *vs* 0%), and pulmonary hypertension (21.1% *vs* 6.6%) in the AC cohort compared to the non-AC cohort ($P < 0.05$) (Table 2). The additional comorbidity burden in the matched cohort is outlined in Supplementary Table 1. AC had no significant association with acute kidney injury (OR 0.89, 95%CI 0.74–1.07, $P = 0.23$) or acute liver failure (OR = 0.97, 95%CI: 0.21-4.34, $P = 0.91$).

On multivariate regression, significant predictors of mortality for the matched cohort included cardiac arrhythmias (OR = 2.34, 95%CI: 1.38-3.97, $P = 0.002$), coagulopathy (OR = 1.87, 95%CI: 1.28-2.73, $P = 0.001$), protein-calorie malnutrition (OR = 5.96, 95%CI: 3.67-9.68, $P < 0.001$), fluid and electrolyte disorders (OR = 1.56, 95%CI: 1.05-2.32, $P = 0.027$), septic shock (OR = 18.77, 95%CI: 10.02-35.13, $P < 0.001$), atrial fibrillation (OR = 2.01, 95%CI: 1.11-3.63, $P = 0.020$), upper extremity VTE (OR = 11.38, 95%CI: 3.65-35.46, $P < 0.001$), and increasing age (OR = 1.06, 95%CI: 1.04-1.07, $P < 0.001$) (Table 3).

DISCUSSION

This national population-based study evaluated hospitalized COVID-19 patients with and without AC according to age, gender, and race to identify high-risk individuals. Our findings indicate that AC does not significantly increase mortality among patients hospitalized with COVID-19. However, it is associated with a higher prevalence of inpatient complications, particularly in certain demographic groups and people with higher ECI scores. Interestingly, despite these complications, there was no significant difference in the need for ICU-level care, length of stay, or hospital charges between the AC and non-AC groups.

Alcohol consumption increased during the COVID-19 pandemic due to isolation and social distancing protocols[23]. A study conducted in the United States analyzed changes in adult alcohol consumption during the pandemic[24]. It reported a 14% increase in the frequency of alcohol use[24]. The median age group in their analysis that saw increased alcohol consumption was similar to the median age in our AC cohort (IQR: 54-67). The increase in alcohol intake during the COVID-19 pandemic in this age group might have aggravated the disease burden of AC[24]. A multicenter study in the United States reported that Hispanic ethnicity and decompensated cirrhosis were associated with a higher risk for severe COVID-19[17]. In our data, we also observed a higher prevalence of Hispanics in the AC cohort than in the non-AC cohort. It is known that increasing age reduces the ability to metabolize alcohol due to decreased mitochondrial function [25]. Upon multivariate analysis of our data, we identified increasing age as a mortality predictor among COVID-19

Table 1 Baseline biodemographic characteristics of hospitalized coronavirus disease 2019 patients with and without alcohol-associated cirrhosis, *n* (%)

Factor	Alcoholic cirrhosis	No alcoholic cirrhosis (before matching)	*P* value	No alcoholic cirrhosis (after matching)	*P* value
Total hospitalizations	1325	736685		1135	
Age groups (yr)			< 0.001		< 0.001
18-34	30 (2.3)	36860 (5.0)		60 (5.3)	
34-49	195 (14.7)	105475 (14.3)		205 (18.1)	
50-64	625 (47.2)	213325 (29.0)		435 (38.3)	
65-79	375 (28.3)	242015 (32.9)		350 (30.8)	
≥ 80	100 (7.5)	139010 (18.9)		85 (7.5)	
Gender			< 0.001		0.31
Male	930 (70.2)	380300 (51.6)		775 (68.3)	
Female	395 (29.8)	356385 (48.4)		360 (31.7)	
Race			< 0.001		< 0.001
White	580 (43.8)	388810 (52.8)		515 (45.4)	
Black	145 (10.9)	129635 (17.6)		180 (15.9)	
Hispanic	440 (33.2)	156285 (21.2)		295 (26.0)	
Asian	25 (1.9)	25065 (3.4)		35 (3.1)	
Native American	65 (4.9)	6500 (0.9)		55 (4.8)	
Other	70 (5.3)	30390 (4.1)		55 (4.8)	
Elixhauser Comorbidity Index score			< 0.001		< 0.001
0	0 (0.0)	44530 (6.0)		25 (2.2)	
1	0 (0.0)	98560 (13.4)		70 (6.2)	
2	15 (1.1)	141150 (19.2)		1040 (91.6)	
≥ 3	1310 (98.9)	452445 (61.4)		25 (2.2)	
Region of hospital			< 0.001		0.008
Northeast	280 (21.1)	133450 (18.1)		245 (21.6)	
Midwest	250 (18.9)	168480 (22.9)		275 (24.2)	
South	430 (32.5)	309115 (42.0)		335 (29.5)	
West	365 (27.5)	125640 (17.1)		280 (24.7)	
Location/teaching status of hospital			< 0.001		0.009
Rural	65 (4.9)	88910 (12.1)		75 (6.6)	
Urban nonteaching	180 (13.6)	147560 (20.0)		115 (10.1)	
Urban teaching	1080 (81.5)	500215 (67.9)		945 (83.3)	
Primary payer			< 0.001		0.030
Medicare	570 (43.0)	369155 (50.1)		455 (40.1)	
Medicaid	420 (31.7)	93610 (12.7)		385 (33.9)	
Private	250 (18.9)	243440 (33.0)		245 (21.6)	
Other	85 (6.4)	30480 (4.1)		50 (4.4)	
Median household income national quartile for patient ZIP code			0.074		0.31
1st (0-25th)	480 (36.2)	249205 (33.8)		445 (39.2)	
2nd (26th-50th)	335 (25.3)	203325 (27.6)		280 (24.7)	

3rd (51st-75th)	310 (23.4)	163695 (22.2)		235 (20.7)	
4th (76th-100th)	200 (15.1)	120460 (16.4)		175 (15.4)	
Disposition of patient			< 0.001		0.024
Discharged to home or self-care (routine discharge)	685 (51.7)	445390 (60.5)		555 (48.9)	
Transfer to short-term hospital	40 (3.0)	21910 (3.0)		20 (1.8)	
Transfer other: Skilled nursing facility, intermediate care facility, or another type of facility	235 (17.7)	98350 (13.4)		205 (18.1)	
Home health care	120 (9.1)	99700 (13.5)		145 (12.8)	
Against medical advice	50 (3.8)	7535 (1.0)		40 (3.5)	
Died during hospitalization	195 (14.7)	63625 (8.6)		170 (15.0)	
Day of admission			0.43		0.27
Weekday	990 (74.7)	543410 (73.8)		870 (76.7)	
Weekend	335 (25.3)	193275 (26.2)		265 (23.3)	
Mechanical ventilation	130 (9.8)	43865 (6.0)	< 0.001	140 (12.3)	0.046
ICU admission	140 (10.6)	43120 (5.9)	< 0.001	120 (10.6)	1.00
Vasopressor requirement	30 (2.3)	9345 (1.3)	< 0.001	30 (2.6)	0.54
Age in years at admission, median (IQR)	60.0 (54.0, 67.0)	65.0 (53.0, 77.0)	< 0.001	61.0 (51.0, 72.0)	0.65
Length of stay in days, median (IQR)	5.0 (3.0, 9.0)	5.0 (3.0, 8.0)	0.016	6.0 (3.0, 11.0)	0.1
Total hospital charges in USD, median (IQR)	44739.0 (24963.0, 80405.0)	39061.0 (22215.0, 71177.0)	< 0.001	49862.0 (25884.0, 90286.0)	0.098

ICU: Intensive care unit; IQR: Interquartile range.

patients with AC. The clinical symptoms between the younger and older age groups might be similar in the presence of AC. However, the elderly tend to develop more complications due to a decline in the robustness of the immune system. In a study conducted at the Johns Hopkins Health System, Krishnan *et al*[26] showed that increasing age and hepatic decompensation were associated with all-cause mortality among COVID-19 patients with CLD.

In our analysis, the mortality rates were comparable in both the AC and non-AC cohorts. One potential explanation could be that during the COVID-19 pandemic, only the most critically ill cirrhotic patients were admitted, rendering the presence of infection negligible in impacting the clinical outcome. Moreover, comorbidities among AC patients with higher ECI scores could affect the mortality rate. This might have resulted in similar ICU admission rates in both AC and non-AC cohorts. Intriguingly, our data showed that more non-AC patients received mechanical ventilation than AC patients. This is noteworthy because it may indicate an attempt to avoid mechanical ventilation, which is commonly regarded as a predictor of death in cirrhosis[10]. Shalimar *et al*[27] showed in their study that the need for mechanical ven -tilation independently predicted mortality in CLD patients with COVID-19.

Cirrhosis-associated immune dysfunction is characterized by systemic inflammation and impaired immunocom-petence[28]. The dysregulated complement factors, immunoglobulins, and acute-phase proteins may correlate with cirr-hosis severity and can serve as biomarkers of immune alterations in these patients[28]. These dysregulations expose cirrhotics to an increased risk of infections and liver-related death[28]. Bolarín *et al*[29] revealed that sepsis was the leading cause of non-sudden death in patients with AC ten years after liver transplantation. Guerra Veloz *et al*[30] showed that hospitalized COVID-19 patients with CLD required almost four times more antibiotic therapy compared to those without CLD. Similar findings were observed in another study, which demonstrated that cirrhotics had an increased risk of mortality, organ dysfunction syndrome, superinfections, and greater influenza severity compared to non-cirrhotics[31]. These observations may explain why a higher prevalence of *Hemophilus* pneumonia, septic shock, and spontaneous bacterial peritonitis was observed among AC patients in our study.

Patients with cirrhosis often develop portal hypertension. It frequently leads to upregulation of angiotensin-converting enzyme 2 (ACE-2) to counteract this major complication of cirrhosis[32]. This makes patients with AC more susceptible to COVID-19 infection, as ACE-2 is the critical functional receptor for SARS-CoV-2[33,34]. Therefore, it could possibly be associated with inpatient complications and a poor prognosis. During the pandemic, hospital stays for patients with cirrhosis were shorter, and more of these patients were discharged to go home compared to the pre-COVID era. Similarly, we observed a higher proportion of AC patients discharged home than non-AC patients in our study. This trend reflects the drive to conserve inpatient resources and promote home isolation unless the patient requires urgent medical treatment. However, it might have increased the risk of early post-hospital discharge mortality in patients with decompensated cirrhosis during the COVID-19 period[35]. A United States national cohort study using data from the Veterans Health Administration reported a decrease in hospitalizations of cirrhotic patients during the pandemic from January to April 2020[36]. Contrarily, in line with the increase in alcohol consumption during the early phase of the

Table 2 Inpatient outcomes of coronavirus disease 2019 patients with and without alcohol-associated cirrhosis, *n* (%)

Variables	Alcoholic cirrhosis	No alcoholic cirrhosis (before matching)	*P* value	No alcoholic cirrhosis (after matching)	*P* value
n	1325	736685		1135	
Asthma exacerbation	5 (0.4)	13170 (1.8)	< 0.001	5 (0.4)	0.81
ARDS	80 (6.0)	31970 (4.3)	0.002	100 (8.8)	0.008
Type I respiratory failure	545 (41.1)	379975 (51.6)	< 0.001	475 (41.9)	0.72
Type II respiratory failure	15 (1.1)	7640 (1.0)	0.73	15 (1.3)	0.67
Bacterial pneumonia	10 (0.8)	6005 (0.8)	0.81	10 (0.9)	0.73
Klebsiella pneumonia	0 (0.0)	1425 (0.2)	0.11	0 (0.0)	-
Streptococcus pneumonia	0 (0.0)	805 (0.1)	0.23	0 (0.0)	-
Staphylococcus pneumonia	5 (0.4)	3850 (0.5)	0.46	10 (0.9)	0.11
Hemophilus pneumonia	5 (0.4)	300 (< 1)	< 0.001	0 (0.0)	-
Anosmia	0 (0.0)	1240 (0.2)	0.14	0 (0.0)	-
Hemorrhagic CVA	0 (0.0)	615 (0.1)	0.29	0 (0.0)	-
Ischemic CVA	0 (0.0)	475 (0.1)	0.36	0 (0.0)	-
Dysgeusia	0 (0.0)	695 (0.1)	0.26	0 (0.0)	-
Diarrhea	80 (6.0)	44110 (6.0)	0.94	65 (5.7)	0.74
Septic shock	75 (5.7)	21780 (3.0)	< 0.001	47 (4.1)	0.007
SVT	30 (2.3)	9375 (1.3)	0.001	25 (2.2)	0.92
VT	20 (1.5)	10065 (1.4)	0.65	40 (3.5)	0.001
Vfib/Vflutter	5 (0.4)	1315 (0.2)	0.087	0 (0.0)	0.038
Afib	175 (13.2)	77340 (10.5)	0.001	100 (8.8)	< 0.001
Aflutter	115 (8.7)	47410 (6.4)	< 0.001	50 (4.4)	< 0.001
First-degree AV nodal block	10 (0.8)	3250 (0.4)	0.086	0 (0.0)	0.003
Second-degree AV nodal block	0 (0.0)	1515 (0.2)	0.098	0 (0.0)	-
Complete AV nodal block	0 (0.0)	1375 (0.2)	0.12	10 (0.9)	< 0.001
ECMO	0 (0.0)	120 (< 1)	0.64	0 (0.0)	-
Total acute VTE	50 (3.8)	26120 (3.5)	0.65	75 (6.6)	0.001
Portal venous thrombosis	5 (0.4)	190 (< 1)	< 0.001	15 (1.3)	0.009
Budd Chiari	0 (0.0)	35 (< 1)	0.80	0 (0.0)	-
Upper extremity VTE	20 (1.5)	1960 (0.3)	< 0.001	0 (0.0)	< 0.001
Lower extremity VTE	10 (0.8)	8050 (1.1)	0.24	10 (0.9)	0.73
Other VTE	10 (0.8)	1285 (0.2)	< 0.001	5 (0.4)	0.32
Pulmonary embolism	20 (1.5)	18055 (2.5)	0.027	50 (4.4)	< 0.001
Variceal bleeding	50 (3.8)	100 (< 1)	< 0.001	0 (0.0)	< 0.001
Hepatorenal syndrome	5 (0.4)	140 (< 1)	< 0.001	15 (1.3)	0.009
Hyponatremia	325 (24.5)	123080 (16.7)	< 0.001	285 (25.1)	0.74
Pulmonary hypertension	280 (21.1)	1210 (0.2)	< 0.001	75 (6.6)	< 0.001
Spontaneous bacterial peritonitis	15 (1.1)	35 (< 1)	< 0.001	5 (0.4)	0.057
Acute liver failure	4 (0.34)	303 (0.45)	< 0.001	4 (0.30)	0.82
New HD	2 (0.15)	750 (0.11)	< 0.001	0 (0)	0.1

ARDS: Acute respiratory distress syndrome; CVA: Cerebrovascular accident; SVT: Supraventricular tachycardia; VT: Ventricular tachycardia; Vfib: Ventricular fibrillation; Vflutter: Ventricular flutter; Afib: Atrial fibrillation; AV: Atrioventricular; ECMO: Extracorporeal membrane oxygenation; VTE: Venous thromboembolism; HD: Hemodialysis.

Table 3 Inpatient mortality predictors for coronavirus disease 2019 patients with alcohol-associated cirrhosis (after matching)

Variables	Odds ratios	*P* value
Alcohol-associated cirrhosis	0.82 (0.64-1.05)	0.12
Cardiac arrhythmias	2.34 (1.38-3.97)	0.002
Coagulopathy	1.87 (1.28-2.73)	0.001
Protein-calorie malnutrition	5.96 (3.67-9.68)	< 0.001
Fluid and electrolyte disorders	1.56 (1.05-2.32)	0.027
Septic shock	18.77 (10.02-35.13)	< 0.001
Atrial fibrillation	2.01 (1.11-3.63)	0.020
Spontaneous bacterial peritonitis	4.28 (0.91-20.1)	0.065
Upper extremity venous thromboembolism	11.38 (3.65-35.46)	< 0.001
Increasing age	1.06 (1.04-1.07)	< 0.001

pandemic, we might foresee a rise in long-term morbidity and mortality related to alcohol-associated liver disease[37]. Therefore, AC hospitalizations may increase in the future, requiring high-level hepatology care and follow-up.

In patients with cirrhosis, the risk of developing VTE is significantly increased due to the reduced ability to synthesize anticoagulation factors[38]. In line with these findings, we also noted a higher prevalence of VTE in the AC cohort. In addition, the presence of VTE was identified as a mortality predictor. Therefore, cirrhotic patients should undergo a case-by-case consideration of thromboprophylaxis for deep vein thrombosis[39]. Another significant variable observed in our data was the higher prevalence of atrial fibrillation among the AC cohort. A nationwide study conducted in Korea demonstrated a 46% increased risk of developing atrial fibrillation in cirrhotic patients compared to the non-cirrhotic control group[40]. Furthermore, with COVID-19 in these patients, there is a higher chance of observing electrocardiographic abnormalities. In a systematic review, a fourfold higher risk of death was reported among COVID-19 patients with atrial fibrillation[41]. This finding overlaps with our data, where atrial fibrillation was also identified as a mortality predictor. Upon multivariate analysis, we also identified cardiac arrhythmias and coagulopathy as mortality predictors for the AC cohort. Therefore, cirrhotic patients must be carefully monitored before being discharged home to prevent such serious complications. Notably, the clinical management of cirrhotics with COVID-19 is complicated because most pharmacological agents are metabolized by cytochrome P450 monooxygenases in the liver[42]. Therefore, patients with COVID-19 may have a higher risk of developing hepatotoxicity in the presence of CLD due to drug-drug interactions [42]. This might have contributed to the overall ICU admission rate reported in the AC cohort in our study.

Recent research revealed several associations between SARS-CoV-2 infection and medical conditions that may lead to liver dysfunction[43]. Moreover, studies have demonstrated that there is an increased risk of decompensation and mortality in COVID-19 patients with pre-existing cirrhosis[44]. This can possibly be attributed to cirrhosis-related immunological modulation, insufficient physiological reserves, and an increased risk of severe COVID-19 disease[43,44]. The underlying mechanism of liver injury secondary to COVID-19 is multifactorial. SARS-CoV-2 may induce direct hepatotoxicity *via* cholangiocytes, translocation from the gut to the liver, or indirect liver damage from systemic inflammation, hypoxia, ischemic insult, or drug-induced liver injury[44]. While the expression of ACE-2 receptors on hepatocytes has been reported, a substantial contribution from indirect causes of liver injury has been described in COVID-19 patients [44]. Furthermore, treating severe COVID-19 infection with certain antiviral agents, immunomodulators, and supportive agents may also cause hepatotoxicity[44]. Large registries report a case fatality rate of 38%, rising to 70% in the Child-Pugh C category[7,44]. These findings highlight the need for vigilant pharmacological management. Further research is warranted to evaluate the pathological interplay between AC and COVID-19. It is particularly important to understand the mecha-nisms of liver injury and the impact of COVID-19 on pre-existing liver disease.

Our retrospective study is one of the largest to evaluate the specific clinical impact of AC on COVID-19 hospitalizations. We analyzed the patient outcomes based on comorbidities, mechanical ventilation, ICU admission, and mortality predictors. Given the critical nature of the disease association, a multidisciplinary approach is required to manage hospitalized COVID-19 patients with AC. This study will provide invaluable information with regard to identifying high-risk patients and monitoring the factors associated with the rising prevalence of AC. One of the major strengths of this study is the detailed comparison between COVID-19 patients with and without AC. This allows for a better understanding of the variables associated with mortality among the two cohorts.

Limitations

We acknowledge certain limitations to our study. The ICD-10 coding system may present inaccurate data when utilizing a large database like NIS, which may potentially skew the analysis. In addition, the NIS data might only be representative of those hospitals participating in the Healthcare Cost and Utilization Project[45]. The information regarding severity of the disease or treatment is not provided in the NIS database. The COVID-19 waves varied from state to state within the United States. Hence, some areas were more heavily impacted than others. Upon analysis of geographical regions in our data, urban teaching hospitals had a greater number of patients with AC. A nationwide study of hospitalized patients in the United States identified urban hospitals as being associated with a greater risk of infection in cirrhotic patients[46]. These infections included sepsis, pneumonia, and spontaneous bacterial peritonitis[46]. Therefore, the mortality rate might be higher, especially in urban hospitals. Moreover, it can be difficult to diagnose chronic non-AC as COVID-19 may lead to abnormalities in liver function testing in hospitalized patients[47,48].

CONCLUSION

This study has thoroughly analyzed the influence of AC on COVID-19 hospitalizations. Our results indicate that the presence of AC does not significantly impact mortality in COVID-19 patients, warranting further evaluation in a larger cohort. Interestingly, despite the higher ECI scores among the AC cohort, the length of stay and ICU admission rates were comparatively similar across the non-AC cohort. Advanced age was found to be a predictor of death in patients with AC, along with other variables like cardiac arrhythmias, coagulopathy, protein-calorie malnutrition, fluid and electrolyte disorders, septic shock, and upper extremity VTE. Due to the multifactorial nature of hepatic injury in COVID-19, further research will be required to evaluate effective pharmacological treatments in COVID-19 patients with AC. Despite the fact that COVID-19 transmission has slowed down, identifying high-risk groups early on is important. It will make it more convenient for hospitalized COVID-19 patients with AC to receive a tailored medical treatment that could improve their prognosis.

ARTICLE HIGHLIGHTS

Research background

Patients with chronic liver disease (CLD) may be at risk of adverse outcomes following coronavirus disease 2019 (COVID-19). Initial findings from Wuhan indicated potential liver injury in COVID-19 patients. With the growing role of obesity and alcohol, understanding the specific implications of alcohol-associated cirrhosis (AC) for patients with COVID-19 is of paramount clinical importance for prognostication and appropriate therapeutic strategy.

Research motivation

Cirrhosis is typically associated with immune system impairments, which might hinder the ability of patients to combat COVID-19. While several studies have indicated an increased risk of COVID-19 severity in cirrhotic patients, the specific impact of AC on inpatient outcomes remains incompletely defined.

Research objectives

This study primarily aims to assess the impact of AC on inpatient outcomes and mortality rates in patients hospitalized with COVID-19 compared to those without AC. Furthermore, we intend to identify predictors of mortality within the COVID-19 patient cohort with AC.

Research methods

A retrospective cohort study was conducted using the National Inpatient Sample database 2020, focusing on hospitalizations with a primary diagnosis of COVID-19. Two cohorts were established based on the presence or absence of AC. Propensity score matching was used to compare the cohorts and a range of statistical analyses were applied to the data.

Research results

Of the 738010 patients hospitalized with COVID-19 that fulfilled the selection criteria, 1325 with AC were compared to 1135 without AC. It was found that AC did not significantly increase mortality in COVID-19 patients. However, it was linked to a higher prevalence of inpatient complications, especially in certain demographics and those with higher Elixhauser Comorbidity Index scores.

Research conclusions

AC does not increase mortality rates in the context of COVID-19 hospitalizations. However, it is associated with a heightened prevalence of certain inpatient complications. Despite these systemic complications, there was no noticeable difference in intensive care unit requirements, length of stay, or hospital charges between AC and non-AC groups.

Research perspectives

This research provides valuable insights into the implications of AC for COVID-19 patients. It prompts clinicians to conduct future research and delve deeper into understanding the exact mechanisms leading to these complications in COVID-19 patients with AC. Exploring preventive measures or pharmacological treatment adjustments for this vulnerable population may improve patient outcomes.

FOOTNOTES

Author contributions: Inayat F, Ali H, Patel P, Dhillon R, and Afzal A concepted and designed the study, participated in the acquisition of data, interpretation of results, writing of the original draft, and critical revisions of the important intellectual content of the final manuscript; Rehman AU, Afzal MS, Zulfiqar L, Nawaz G, Goraya MHN, Subramanium S, and Agrawal S contributed to the analysis and interpretation of results and drafting of the manuscript; Satapathy SK reviewed, revised, and improved the manuscript by suggesting pertinent modifications; and all authors critically assessed, edited, and approved the final manuscript and are accountable for all aspects of the work.

Institutional review board statement: The data of patients was not acquired from any specific institution but rather open-access United States National Inpatient Sample (NIS) database. The NIS contains de-identified information, protecting the privacy of patients, physicians, and hospitals. Therefore, it was deemed exempt from the institutional review board.

Informed consent statement: Participants were not required to give informed consent for this retrospective cohort study since the analysis of baseline characteristics used anonymized clinical data.

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

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ORIGINAL ARTICLE

Retrospective Study

Performance evaluation of NeuMoDx 96 system for hepatitis B and C viral load

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Abstract

BACKGROUND

Hepatitis B virus (HBV) and hepatitis C virus (HCV) viral load (VL) estimation is essential for the management of both HBV and HCV infections. Due to a longer turnaround time for VL estimation, many patients drop out from the cascade of care. To achieve the global goals of reducing morbidity and mortality due to HBV/HCV and moving towards their elimination by 2030, molecular diagnostic platforms with faster and random (*i.e.* single sample) access are needed.

AIM

To evaluate the performance of the recently launched NeuMoDx 96 random access system with the conventional COBAS®AmpliPrep/COBAS TaqMan system for HBV and HCV VL estimation.

METHODS

Archived once-thawed plasma samples were retrieved and tested on both platforms. Correlation between the assays was determined by linear regression and Bland-Altman analysis. The study included samples from 186 patients, 99 for HBV of which 49 were true infected HBV cases (hepatitis B surface antigen, anti-hepatitis B core antibody, and HBV DNA-positive) and 87 for HCV assay in which

39 were true positives for HCV infection (anti-HCV and HCV RNA-positive).

RESULTS

The median VL detected by NeuMoDx for HBV was 2.9 (interquartile range [IQR]: 2.0-4.3) $\log_{10}$ IU/mL and by COBAS it was 3.70 (IQR: 2.28-4.56) $\log_{10}$ IU/mL, with excellent correlation (R^2 = 0.98). In HCV, the median VL detected by NeuMoDx was 4.9 (IQR: 4.2-5.4) $\log_{10}$ IU/mL and by COBAS it was 5.10 (IQR: 4.07-5.80) $\log_{10}$ IU/mL with good correlation (R^2 = 0.96).

CONCLUSION

The overall concordance between both the systems was 100% for both HBV and HCV VL estimation. Moreover, no genotype-specific bias for HBV/HCV VL quantification was seen in both the systems. Our findings reveal that NeuMoDx HBV and HCV quantitative assays have shown overall good clinical performance and provide faster results with 100% sensitivity and specificity compared to the COBAS AmpliPrep/COBAS TaqMan system.

Key Words: Hepatitis B; Hepatitis C; NeuMoDx; Random access; Viral load; COBAS AmpliPrep

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Core Tip: In this study, the clinical performance of the NeuMoDx 96 random access system for hepatitis B virus (HBV) and hepatitis C virus (HCV) viral load (VL) quantification was evaluated and compared to the routinely used COBAS AmpliPrep/COBAS TaqMan system. Good concordance was observed between both systems for the HBV and HCV VL assays. Due to its random access (*i.e.* single sample access) characteristics and shorter turnaround, the NeuMoDx 96 can be considered a faster and effortless molecular testing system.

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INTRODUCTION

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are among the most common causes of chronic liver diseases worldwide. This underscores its public health significance, with more than 250 million HBV carriers and about 70 million people with chronic HCV infection[1]. As a result of HBV vaccination programs[2,3] and direct-acting antiviral treatment against HCV[4], the prevalence of these infections has significantly decreased but both are still major risk factors for the development of cirrhosis and hepatocellular carcinoma. In resource-poor nations, gaps in HBV and HCV screening, treatment, and monitoring, as well as a lack of efficient therapeutic management choices for HBV/HCV-related liver cancer, have resulted in a low survival rate among infected persons. In response to this significant worldwide public health burden, the World Health Organization launched a worldwide Health Sector Strategy on Hepatitis 2016-2021 in 2016, with the objective of eliminating viral hepatitis as a public health issue by 2030[5]. Moreover, HBV and HCV account for about 96% of all viral hepatitis deaths in low- and middle-income countries[1]. The complete burden of disease includes not just death but also decreased quality of life for patients (due to cirrhosis and associated comorbidities), financial expenses of care for people and health care systems alike, and social economic costs[6]. Serological assays to detect antigens/antibodies to HBV/HCV do not differentiate between an active and past infection; thus, an active ongoing infection must be confirmed by the detection of viral DNA/RNA[7]. This two-step process for diagnosing active HBV & HCV infection, *i.e.* first a serological test to screen for exposure, followed by a nucleic acid test (NAT) to confirm viremia often takes a longer time and leads to patient loss to follow-up[6].

Therefore, to prevent loss to follow-up of a patient from the cascade of care, it is important to have molecular assays that are fully automated with high throughput and short turnaround (TAT), particularly in low-income countries[8].

At present, batch-based closed molecular testing platforms are routinely being used but recently fully automated random access (*i.e.* single sample) closed systems are coming up and have demonstrated good potential[9,10]. The NeuMoDx 96 molecular system (QIAGEN Sciences, Waltham, MA, United States) is one such recent fully automated random access closed system. Therefore, the present study compared and evaluated the clinical performance of the NeuMoDx 96 molecular system (referred to as assay 1) for HBV and HCV viral load (VL) testing with the routinely used COBAS®AmpliPrep/COBAS® TaqMan®, v2.0 (Roche Diagnostics, GmbH, Mannheim, Germany) system (referred to as assay 2).

MATERIALS AND METHODS

Study population

This was a retrospective study conducted in a tertiary care liver center in Delhi, India. A total of 186 archived once-thawed plasma samples with pre-existing test results from initial routine lab testing were used. Among these, 99 samples for the HBV assay and 87 samples for the HCV assay were simultaneously tested on both platforms from the same freeze-thaw cycle. Of the 99 samples, 49 were HBV true positive (hepatitis B surface antigen [HBsAg], anti-hepatitis B core antibody [anti-HBc] total, and HBV DNA-positive; VL range: 1 to > 10^6 $\log_{10}$ IU/mL), and the remaining 50 were confirmed negatives for HBV (HBsAg, anti-HBc and HBV DNA-negative). In the HCV assay, of the 87 samples, 39 were true positives for HCV infection (anti-HCV antibody and HCV RNA-positive; VL range: 1 to > 10^6 $\log_{10}$ IU/mL), and the remaining 48 were confirmed negatives for HCV infection (anti-HCV antibody and HCV RNA negative).

The clinical details along with other virological parameters were obtained from our Hospital Information System. Samples from patients with other co-infections, such as human immunodeficiency virus and hepatitis delta virus, on immunosuppressants and samples with insufficient available volume were excluded. This study was approved by the Institutional Ethics Board (Approval No. IEC/2023/102/MA06) and conducted as per the principles of the Declaration of Helsinki. The study was performed on deidentified, anonymous, archived once-thawed clinical plasma samples. Therefore, the requirement of individual patient consent was waived.

NeuMoDx 96 molecular system (assay 1)

In the NeuMoDx 96, 550 µL plasma was used in the instrument as per the manufacturer's instructions. The results of the test were automatically displayed on the system approximately within 1 h for HBV DNA and approximately within 1.25 h for HCV RNA. The results are expressed as IU/mL. The lower limit of detection (LOD) and lower limit of quantification (LLOQ) for both HBV and HCV assays provided by the manufacturer are 8.0 IU/mL and 0.9 $\log_{10}$ IU/mL, respectively (Table 1).

COBAS®AmpliPrep/COBAS® TaqMan® (assay 2)

In the COBAS®AmpliPrep/COBAS® TaqMan®, v2.0, 650 µL plasma sample was used in the instrument. The results of the test were automatically displayed on the system within 5 h for HBV DNA and about 5 h 45 min for HCV RNA. For the HBV assay, the LOD and LLOQ claimed by the manufacturer are 20 IU/mL and 1.3 $\log_{10}$ IU/mL, respectively. For the HCV assay, the LOD and LLOQ are ≥ 15 IU/mL and 1.5 $\log_{10}$ IU/mL, respectively (Table 1).

Statistical analyses

The HBV and HCV VLs are expressed in $\log_{10}$ format. Continuous variables were summarized using the mean ± SD or median with interquartile range (IQR), as applicable. Categorical variables are presented as percentages. Linear regression and Pearson's correlation coefficient were determined for VL results between assay 1 and assay 2. The Bland-Altman plot was used to determine the level of agreement between the assays, in which the difference in HBV VLs measured by the two assays was plotted against the mean of the assays. All analyses were done using Statistical Package for the Social Sciences software version 22 (SPSS Inc., Chicago, IL, United States).

RESULTS

Characteristics of the study population

The baseline demographic and clinical characteristics of the study population are described in Table 2. The male:female ratio was 2.5:1.0. The overall mean age was 45 years ± 15 years. The median VL detected for the HBV DNA-positive samples was 2.9 (IQR 2.0-4.3) $\log_{10}$ IU/mL and 3.7 (IQR: 2.28-4.56) $\log_{10}$ IU/mL for assay 1 and assay 2, respectively. For HCV RNA, the median VL detected was 4.9 (IQR: 4.2-5.4) $\log_{10}$ IU/mL and 5.1 (IQR: 4.07-5.80) $\log_{10}$ IU/mL for assay 1 and assay 2, respectively. Further, for the HBV assay, genotyping data were available for 24 plasma samples with the following information: GTD (n = 10 as D1 and n = 8 as D2), GTA (n = 4, all as A1) and GTC (n = 2, all as C1). For the HCV assay, genotype results were available for 34 plasma samples with the following distribution: GT3 (n = 20 as 3a and n = 4 as 3b), GT1 (n = 6, all were GT1a), and GT4 (n = 4, all were GT4c).

Clinical performance of assay 1 for HBV VL

A total of 99 samples were analyzed, of which 49 were true HBV positives and 50 were true negatives for HBV infection. A 100% concordance was observed for both positive and negative samples. Therefore, the sensitivity (95% confidence interval [CI]: 92.75-100.00) and the specificity (95%CI: 92.89-100.00) were found to be 100%. Assay 1 showed no difference in quantification of HBV VL across different genotypes compared to assay 2.

Correlation and agreement analyses of HBV VL measurement

A total of 49 confirmed HBV DNA-positive samples were used with VL ranging from 1.0 to 7.7 $\log_{10}$ IU/mL (Table 3). Linear regression analysis of the quantifiable VLs obtained by both assays demonstrated a good correlation (Pearson's correlation coefficient; r^2 = 0.991) (Figure 1A). The Bland-Altman plot was used to determine the level of agreement between the assays (Figure 1B). The mean difference between assay 1 and assay 2 was 0.05 $\log_{10}$ IU/mL, with agreement limits ranging from -0.458 to 0.694 $\log_{10}$ IU/mL. With an intraclass correlation coefficient (ICC) of 0.995 (95%CI: 0.990-

Table 1 Comparison of characteristics between assay 1 and assay 2

Parameter	HBV		HCV	
	Assay 1	Assay 2	Assay 1	Assay 2
Sample volume	550 µL plasma	650 µL plasma	550 µL plasma	650 µL plasma
Turnaround time	Approximately 1.00 h	Approximately 5.00 h	Approximately 1.25 h	Approximately 5.45 h
LOD in IU/mL	8.0	20	8.0	≥ 15
LLOQ as $\log_{10}$ IU/mL	0.9	1.3	0.9	1.5
Linear range quantification as $\log_{10}$ IU/mL	1-9	1.3-8.2	1-9	1.5

HBV: Hepatitis B virus; HCV: Hepatitis C virus; IU: International unit; LLOQ: Lower limit of quantitation; LOD: Limit of detection.

Table 2 Baseline demographic and clinical characteristics of the study population

Characteristic	HBV patient group	HCV patient group
Patients, *n*	99	87
Sex as M/F ratio	3.34:1.00	1.71:1.00
Age in yr, mean ± SD	44.0 ± 14.6	46.0 ± 14.9
Total bilirubin in mg/dL, median (IQR)	0.83 (0.59-0.83)	0.95 (0.58-2.15)
Direct bilirubin in mg/dL, median (IQR)	0.18 (0.10-0.47)	0.25 (0.10-0.62)
ALT values in IU/mL, median (IQR)	32.5 (23.2-60.1)	33 (17.0-59.7)
AST values in IU/mL, median (IQR)	36 (28.00-62.75)	46 (27.0-99.2)
NeuMoDx viral load as $\log_{10}$ IU/mL, median (IQR)	2.9 (2.0-4.3)	4.9 (4.2-5.4)
COBAS viral load as $\log_{10}$ IU/mL, median (IQR)	3.7 (2.28-4.56)	5.1 (4.07-5.80)

ALT: Alanine transaminase, AST: Aspartate aminotransferase; F: Female; HBV: Hepatitis B virus; HCV: Hepatitis C virus; IQR: Interquartile range; IU: International unit; M: Male; SD: Standard deviation.

Table 3 Comparison of hepatitis B virus viral load results between assay 1 and assay 2

Range of viral load as $\log_{10}$ IU/mL	HBV	
	Assay 1, *n* = 49	Assay 2, *n* = 49
10^1-10^3	*n* = 25 (51.0%)	*n* = 19 (38.7%)
10^3-10^6	*n* = 18 (36.7%)	*n* = 25 (51.0%)
> 10^6	*n* = 6 (12.2%)	*n* = 5 (10.2%)

HBV: Hepatitis B virus.

0.997; P = 0.001), the two systems agreed well (Figure 1B).

Clinical performance of assay 1 for HCV VL

A total of 87 samples were analyzed, of which 39 were true HCV positives and 48 were confirmed negatives for HCV infection. A 100% concordance was observed for both the positive and negative samples. Therefore, the sensitivity (95%CI: 90.97-100.00) and specificity (95%CI: 92.6-100.0) were found to be 100% compared to assay 2. No difference was seen in VL quantification among the different HCV genotypes between assay 1 and assay 2.

Correlation and agreement analyses of HCV VL measurement

A total of 39 confirmed HCV RNA-positive samples were used with VL ranging from 1.26 to 7.09 $\log_{10}$ IU/mL (Table 4). Linear regression analysis of the quantifiable VLs obtained by both assays demonstrated a good correlation (Pearson's correlation coefficient; r^2 = 0.978) (Figure 1C). The Bland-Altman plot was used to determine the level of agreement between the assays (Figure 1C). The mean difference between assay 1 and assay 2 was 0.06 $\log_{10}$ IU/mL, with agreement

Table 4 Comparison of hepatitis C virus viral load results between assay 1 and assay 2

Range of viral load (log_{10} IU/mL)	HCV	
	Assay 1 (*n* = 39)	Assay 2 (*n* = 39)
10^1-10^3	*n* = 4 (10.2%)	*n* = 2 (5.1%)
10^3-10^6	*n* = 32 (82.0%)	*n* = 28 (71.7%)
> 10^6	*n* = 3 (7.69%)	*n* = 9 (23.07%)

HCV: Hepatitis C virus.

limits ranging from -0.987 to 1.182 log_{10} IU/mL. With an ICC of 0.988 (95%CI: 0.981-0.992; *P* = 0.001), the two systems agreed well (Figure 1D).

DISCUSSION

In this study, the performance of a random access system (NeuMoDx 96) was compared with the currently used batch system (COBAS AmpliPrep/COBAS TaqMan) for the detection of HBV DNA and HCV RNA from clinical samples. A concordance of 100% was observed between both systems for both HBV DNA and HCV RNA analyses. Moreover, both assays demonstrated a good correlation with respect to the VL estimation. Such systems with random access to samples are the need of the hour, so that results are provided in a shorter TAT to the clinicians[11]. In the cascade of care for both HBV DNA and HCV RNA, patients screened initially through serological tests often are lost to follow-up for VL testing due to either non-availability of the assays or a high TAT. The use of existing batch testing platforms across most of the diagnostic labs results in longer "sample-to-result" time[12-15].

At present, for conventional real-time PCR, closed systems are commonly used[16]. Though the analytical sensitivity or LOD of these assays is good and lies between 5 and 15 IU/mL, these available assays typically run on batch testing to justify and minimize the cost of the test. This further leads to an increase in TAT, which eventually delays the test results, and augments the total number of visits of the patients to the health care facility, and dropouts from the care process[17]. Another important aspect is that if viremic patients are not identified earlier, they pose a significant risk of transmission of active infection to their family members and close contacts due to the nature of transmission of both HBV and HCV infections.

Almost near point-of-care molecular testing platforms that are available at present for HBV/HCV VL are very limited (GeneXpert, TrueNAT)[9,18]. These systems provide random access to samples but are useful in diagnostic sites with low sample throughput such as in peripheral settings or remote areas, whereas systems such as NeuMoDx can cater to large numbers of samples and be placed easily in a high-throughput laboratory. Molecular testing platforms such as NeuMoDx can provide faster results with a very short TAT in a cost-effective manner. Limited studies regarding the evaluation of the NeuMoDx platform are available. Our study focused on its real-life evaluation on clinical samples[19-21].

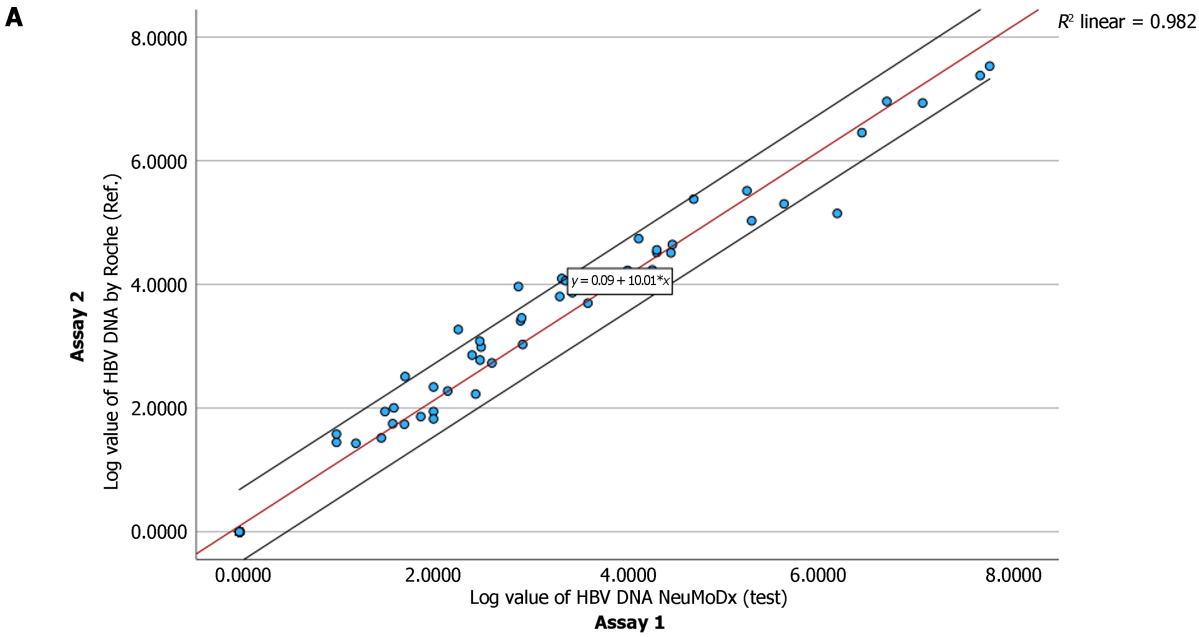
We found good correlation of HBV DNA and HCV RNA levels, especially when the VL in the sample was higher (> 10^6 log_{10} IU/mL). However, in VL 10^1-10^3 log_{10} IU/mL, despite good concordance, only satisfactory correlation was seen. This could be because very low VLs in clinical samples are often difficult to quantitate in terms of their exact values.

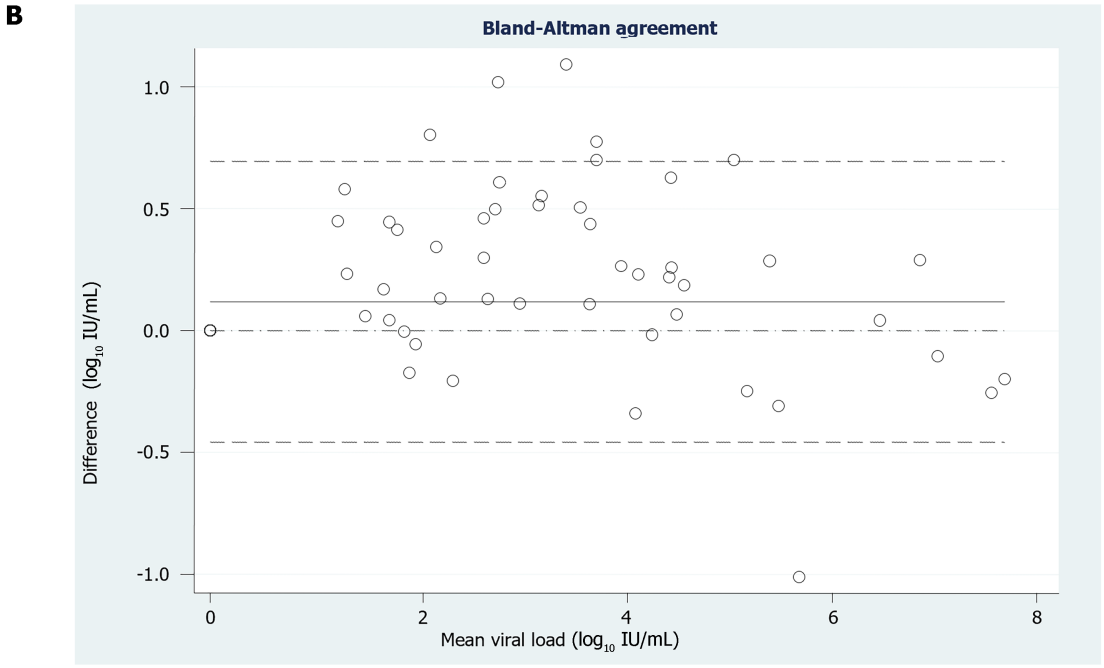
This system includes various functions including specimen preparation, nucleic acid extraction, real-time PCR set-up, amplification, and detection, all integrated within a single platform. Notably, this system provides numerous advantages, including high-throughput capacity, complete traceability, minimal risk of cross-contamination, and stringent safety standards for end users. The assay holds all of the reagents required for up to 20 different assays on board and works at room temperature. Therefore, this automated assay can be used in resource-limited settings, particularly in areas where refrigeration could be a problem. This can be used just like chemiluminescence platforms used for serological testing and would not require specialized molecular testing facilities or separate infra-structure requirements[22], which are extremely expensive to design and require specialized trained manpower to perform such assays. The expertise needed to perform testing on this platform and subsequent manual steps are greatly reduced and a single person can operate on the machine and perform multiple assays together.

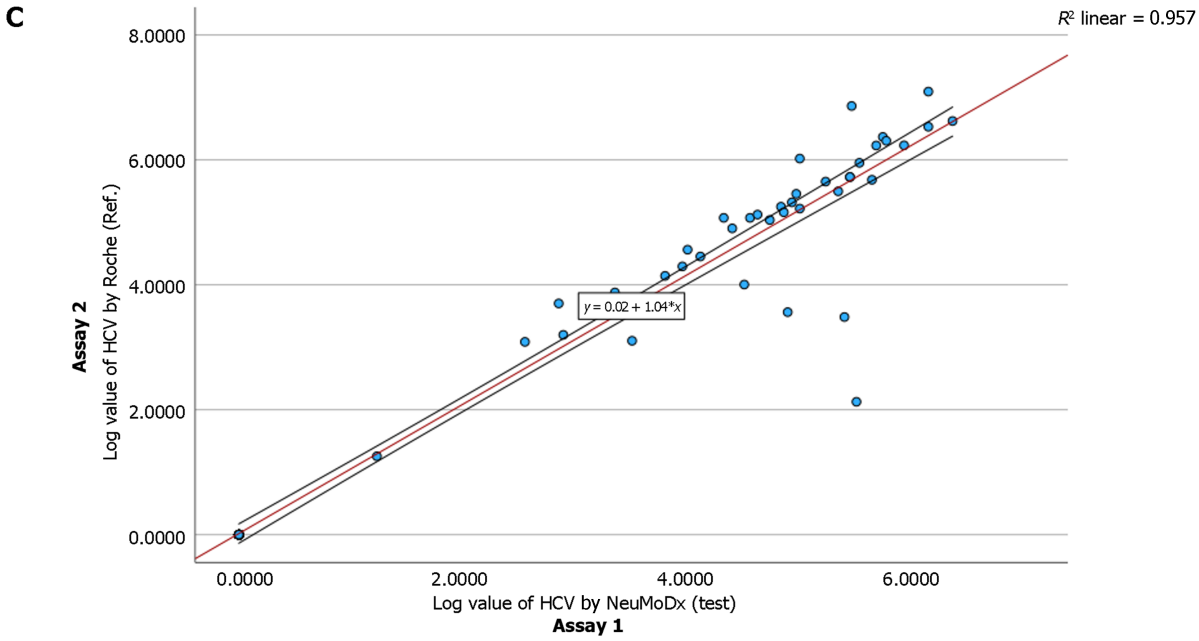
An important limitation of our study is that it was a retrospective, single-center study done on already archived tested samples; thus, large-scale prospective studies in real time are needed to further evaluate their reproducibility, stability, and cost-effectiveness.

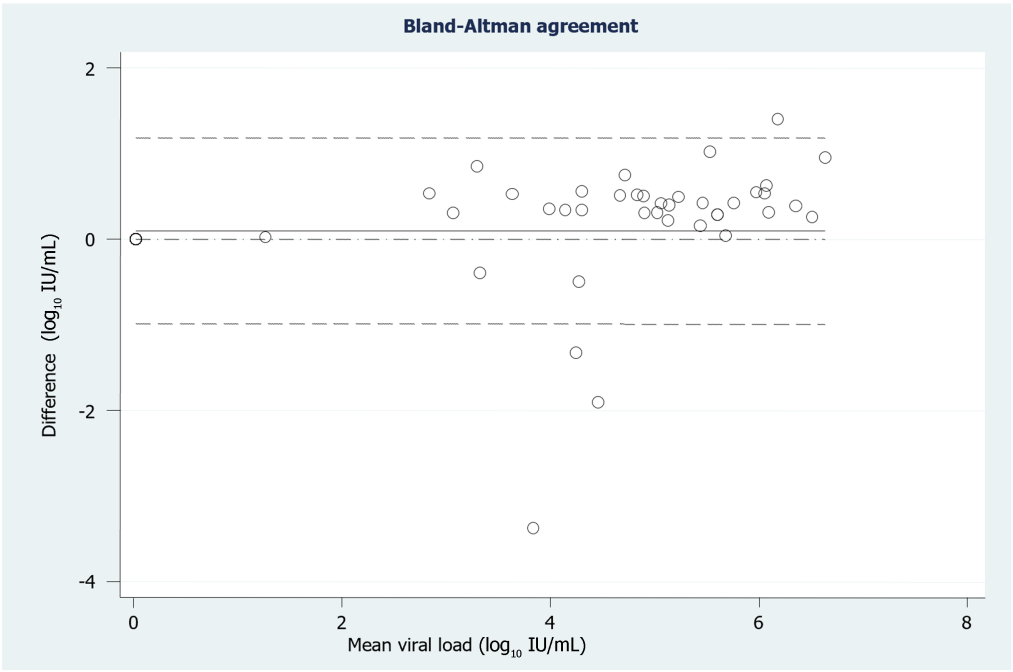
CONCLUSION

The findings of the study demonstrated excellent clinical performance (100% concordance) of the random access system compared to the conventional routinely used batch system. With a short TAT and user-friendly operation, it is a reliable assay for HBV and HCV VL assessment. These promising preliminary results indicate that the NeuMoDx 96 system holds great potential as a novel VL measurement solution in effective patient care and management.


A
R² linear = 0.982
y = 0.09 + 10.01*x
Log value of HBV DNA by Roche (Ref.)
Assay 2
Log value of HBV DNA NeuMoDx (test)
Assay 1
0.0000
2.0000
4.0000
6.0000
8.0000


B
Bland-Altman agreement
Difference (log10 IU/mL)
Mean viral load (log10 IU/mL)
1.0
0.5
0.0
-0.5
-1.0
0
2
4
6
8


C
R² linear = 0.957
y = 0.02 + 1.04*x
Log value of HCV by Roche (Ref.)
Assay 2
Log value of HCV by NeuMoDx (test)
Assay 1
0.0000
2.0000
4.0000
6.0000
8.0000



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Figure 1 Linear regression analysis and Bland-Altman plot. A: Linear regression analysis for correlation of hepatitis B virus (HBV) viral load between assay 1 and assay 2; B: Bland-Altman plot for comparison of HBV viral load between assay 1 and assay 2; C: Linear regression analysis for correlation of hepatitis C virus (HCV) viral load between assay 1 and assay 2; D: Bland-Altman plot for comparison of HCV viral load between assay 1 and assay 2.

ARTICLE HIGHLIGHTS

Research background

Large-scale prospective studies are needed to evaluate the overall analytical, clinical performance, and reproducibility of the NeuMoDx 96 system.

Research motivation

The findings of this study demonstrated excellent clinical performance (100% concordance) of the random access system compared to the conventional commercially available batch system. With a short turnaround time (TAT) and user-friendly operation, it is a reliable assay for hepatitis B virus (HBV) and hepatitis C virus (HCV) viral load (VL) assessment.

Research objectives

In this study, overall a good correlation (100% concordance) and agreement were found between the two systems for HBV DNA and HCV RNA VL quantification. The sensitivity and specificity of the NeuMoDx 96 for both HBV and HCV assays were found to be 100%. Moreover, no difference was found in the quantification of HBV/HCV VL across different genotypes.

Research methods

A total of 186 archived once-thawed plasma samples with pre-existing test results from initial routine lab testing were used. The overall concordance, correlation, and agreement were evaluated among all samples for HBV and HCV VL estimation using both systems.

Research results

The objectives of the study were: (1) Comparison of a random access system with conventional routinely used real-time PCR for quantifying HBV and HCV VL in plasma samples; (2) to estimate the overall concordance and agreement of VL quantification of clinical samples between the two systems; and (3) to evaluate genotype-based comparison of HBV and HCV VL between both systems.

Research conclusions

To date, most routinely used assays for HBV/HCV VL estimation typically run on batch testing. The use of such platforms across most of the diagnostic lab results in longer "sample-to-result" time, leading to loss to follow-up. Therefore, a reliable random access system with a shorter TAT is the need of the hour for all clinicians.

Research perspectives

HBV and HCV pose a significant health burden in low- and middle-income countries. The TAT for VL quantification (from receiving samples to giving out results) is longer for the conventional routinely used batch system-based real-time PCR assays. Therefore, to prevent loss to follow-up of a patient from the cascade of care, it is important to have molecular assays that are fully automated with high throughput and short TAT.

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The primary aim of *World Journal of Virology* (*WJV*, *World J Virol*) is to provide scholars and readers from various fields of virology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJV mainly publishes articles reporting research results obtained in the field of virology and covering a wide range of topics including arbovirus infections, viral bronchiolitis, central nervous system viral diseases, coinfection, DNA virus infections, viral encephalitis, viral eye infections, chronic fatigue syndrome, animal viral hepatitis, human viral hepatitis, viral meningitis, opportunistic infections, viral pneumonia, RNA virus infections, sexually transmitted diseases, viral skin diseases, slow virus diseases, tumor virus infections, viremia, and zoonoses.

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REVIEW

Emerging perspectives on RNA virus-mediated infections: from pathogenesis to therapeutic interventions

Pratik Mohanty, Poojarani Panda, Rakesh Kumar Acharya, Babita Pande, LVKS Bhaskar, Henu Kumar Verma

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Abstract

RNA viruses continue to pose significant threats to global public health, necessitating a profound understanding of their pathogenic mechanisms and the development of effective therapeutic interventions. This manuscript provides a comprehensive overview of emerging perspectives on RNA virus-mediated infections, spanning from the intricate intricacies of viral pathogenesis to the forefront of innovative therapeutic strategies. A critical exploration of antiviral drugs sets the stage, highlighting the diverse classes of compounds that target various stages of the viral life cycle, underscoring the ongoing efforts to combat viral infections. Central to this discussion is the exploration of RNA-based therapeutics, with a spotlight on messenger RNA (mRNA)-based approaches that have revolutionized the landscape of antiviral interventions. Furthermore, the manuscript delves into the intricate world of delivery systems, exploring innovative technologies designed to enhance the efficiency and safety of mRNA vaccines. By analyzing the challenges and advancements in delivery mechanisms, this review offers a roadmap for future research and development in this critical area. Beyond conventional infectious diseases, the document explores the expanding applications of mRNA vaccines, including their promising roles in cancer immunotherapy and personalized medicine approaches. This manuscript serves as a valuable resource for researchers, clinicians, and policymakers alike,

offering a nuanced perspective on RNA virus pathogenesis and the cutting-edge therapeutic interventions. By synthesizing the latest advancements and challenges, this review contributes significantly to the ongoing discourse in the field, driving the development of novel strategies to combat RNA virus-mediated infections effectively.

Key Words: RNA virus; Infections; Therapeutics; Drug target; Pathogenesis

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Core Tip: This comprehensive review explores the intricate world of RNA viruses, highlighting innovative antiviral drugs, mRNA-based therapies, and advanced delivery systems. Our research delves into the nuances of viral pathogenesis and offers insights into combating infections. By synthesizing the latest advancements, this manuscript is a valuable resource for researchers, clinicians, and policymakers. We believe our work significantly contributes to the ongoing discourse and development of novel strategies against RNA virus-mediated infections.

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INTRODUCTION

Viruses are minute parasitic particles available in various shapes and sizes. These viral particles contain genetic material in the form of RNA or deoxyribonucleic acid (DNA), which can be single-stranded (ss) or double-stranded (ds), enclosed within a viral-encoded proteinaceous capsid coat. RNA viruses, characterized by exceptionally high genetic variability and phenotypic diversity, are intracellular obligatory parasites, capable of infecting a wide range of hosts[1,2]. They primarily target Eukarya and replicate using virally encoded RNA-dependent RNA polymerase (RdRp). In this type of viral replication, the synthesized RNA can serve as the genome, a copy of the genome, or messenger RNAs (mRNAs)[3]. Depending on the type of RNA acting as the genome, RNA viruses can be positive or plus strand or negative or minus strand.

RNA viruses are responsible for recurrent epidemics and occasional pandemics. Infections caused by respiratory and vector-borne RNA viruses, such as Influenza A virus, Zika virus, and West Nile virus, are of significant concern[4-6]. Other pathogenic RNA viruses affecting humans include Orthomyxoviruses, hepatitis C virus (HCV), Ebola virus, SARS, influenza, poliovirus, measles virus, and retroviruses such as adult human T-cell lymphotropic virus type 1 (HTLV-1) and human immunodeficiency virus (HIV). HIV, the causative agent of acquired immunodeficiency syndrome (AIDS), is a serious and prevalent viral disease. Currently, approximately 39 million people are living with HIV, and tens of millions have succumbed to AIDS since the beginning of the epidemic[7].

Recent outbreaks, such as the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2019, have underscored the importance of understanding the molecular mechanisms governing the evolution of RNA genomes and the potential for viral exposure[8]. Studies in this area have shed light on novel findings related to viral replication, host-virus interactions, and the development of antiviral therapies. Researchers continue to explore these aspects to develop strategies for combating RNA viral infections and mitigating their impact on global health.

RNA viruses are responsible for recurrent epidemics and occasional pandemics, causing significant global human morbidities and mortalities through virally induced emerging infectious diseases. The emergence and re-emergence of these viral infections have profound implications for public health, the overall economy, and the quality of life of affected populations. To mitigate their impact, it is essential to prevent the replication of RNA viruses, including retroviruses. Therefore, a comprehensive understanding of the replication and transcription processes of these pathogens is crucial[9].

Research on RNA viruses and the infections they cause serves as a pivotal foundation for vaccine development and the formulation of strategies for prevention, control, and corresponding therapeutic interventions. A deep comprehension of virus-host interactions is paramount to understanding the mechanisms governing viral replication and the associated pathological consequences. Advances in sequencing methods have been instrumental in revealing the significance of RNA-protein and RNA-RNA interactions during infections, providing valuable insights into the development of targeted therapies[10].

This review article aims to explore the current understanding of various RNA virus infections, focusing on their pathogenesis and the latest therapeutic interventions. Recent research in this field has led to the identification of novel drug targets, the development of antiviral agents, and the exploration of innovative vaccination strategies. Additionally, studies have elucidated the role of host immune responses and the viral factors contributing to disease severity, paving the way for personalized treatment approaches. Understanding the genetic diversity of RNA viruses, their evolutionary dynamics, and the mechanisms of viral transmission is essential for devising effective public health measures and preparedness strategies against future outbreaks. Moreover, ongoing research efforts continue to unravel the intricate interactions between RNA viruses and host cells, providing valuable information for the development of next-generation

antiviral therapies and vaccines.

RNA VIRUS REPLICATION AND TRANSMISSION

The viral DNA is protected and transported from cell to cell by very basic macromolecular structures known as envelopes or capsids in the extracellular environment**.** Only at the intracellular stage of their life viruses can produce distinctive compounds and engage in activities that are unique to living things. Creating a platform for genome replication and morphogenesis is one of these activities[11]. Due to the error-prone nature of RNA-dependent RNA polymerases of RNA viruses, they live as quasispecies with several variants within their populations[12]. A crucial phase in the life cycle of a virus is the replication of its genome. This procedure involves an intermediary complementary RNA strand for both plus- and minus-strand RNA viruses, but DNA for retroviruses[13].

The primary processes and entrance points for both enveloped and non-enveloped viruses are attachment to cell-surface receptors and transport of the viral genome to the host cell's cytoplasm. After attaching to receptors, which might be proteins, carbohydrates, or lipids, viruses enter the cell by one of two routes: endocytic or non-endocytic. After attaching to receptors, which might be proteins, carbohydrates, or lipids, viruses enter the cell by one of two routes: endocytic or non-endocytic[14]. Alphaviruses, Coronaviruses, Picornaviridae enteroviruses, and Flaviviruses are examples of positive-sense RNA (+RNA) viruses that significantly alter cellular membranes to act as platforms for replication and the assembly of new virions[15]. Since viral genomic RNA replication occurs in the cytosol of host cells, viruses must be able to distinguish between their own genome and many cellular RNAs that are present in cells, so that they amplify only their own genome. The co-optation of host RNA-binding proteins by RNA viruses to speed up replication or dodge host RNA breakdown mechanisms is expected[16].

All (+) RNA viruses can sequester host intracellular membranes to produce replication compartments (RCs). These RCs contain recruited host proteins and lipids as well as viral RNA and proteins, which together produce an environment that is favourable for RNA replication[17]. Capsids have not been found in RCs, suggesting that viral RNA is duplicated within RCs and then (+) RNAs are transferred outside to virion assembly sites. Figure 1 depicted the schematic representation of replication and transmission of positive-sense RNA viruses.

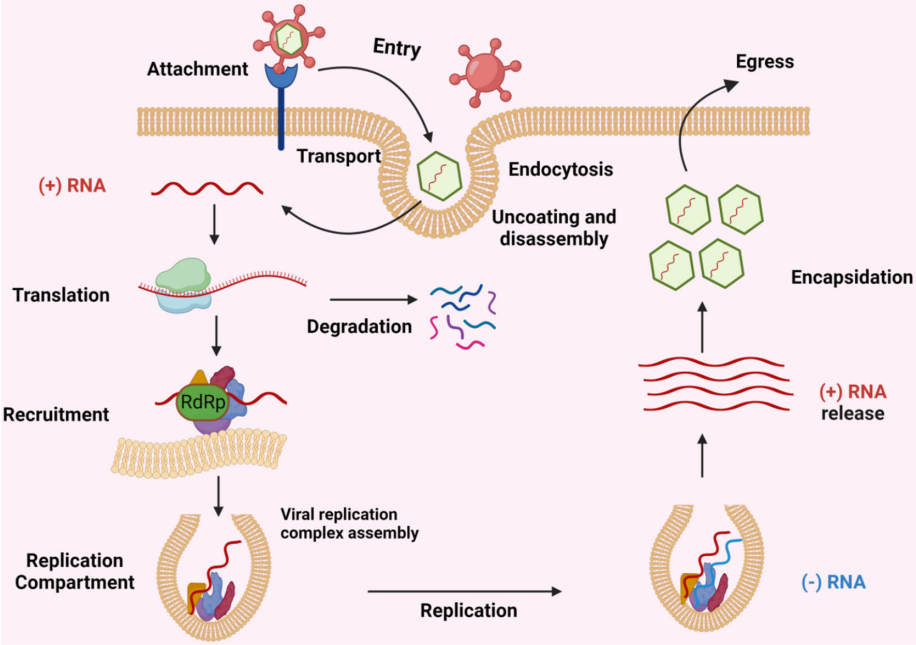
FACTORS CONTRIBUTING TO VIRAL ADAPTABILITY AND EVOLUTION

Similar to animal RNA viruses, plant RNA viruses may have evolved through three different pathways, including transfer of genes horizontally from hosts, parallel evolution with similar genetic components and coevolution or codivergence with hosts[18,19]. Three temporal phases of emergence were identified by Elena *et al*[20-22], these were: The host moves to a different species or the same species but in a different ecological condition, acclimatization to the new environment or host, and epidemiology in the recently arrived host population, often by adjusting to a new vector species or mechanism of transmission. The evolution and host adaptability of animal RNA viruses have piqued the interest of many researchers. The majority of animal RNA viruses have A-rich coding sequences, as reported by Kustin *et al*[23] They also proposed possible explanations such as codon usage bias, weakened RNA secondary structures, and selection for a particular composition of amino acids, concluding that host immunological forces may be the cause of similar biases in the makeup of coding sequences among animal RNA viruses.

ROLE OF HOST FACTORS IN VIRAL REPLICATION AND DISSEMINATION

Various strategies have evolved in (+) RNA viruses to utilize host cell resources. For replication to occur, the viral genomic RNA, along with viral and host components, must be actively attracted to the relevant subcellular membrane surfaces[13]. In the case of human poliovirus (PV), the host poly(rC)-binding protein 2 (PCBP2) plays a crucial role in recruiting RNA templates. PCBP2, an RNA-binding protein, facilitates cap-independent translation by binding to the internal ribosome entry site (IRES) in PV (+)RNA and stabilizing mRNA[20]. Upon binding to viral RNA, PCBP2 is cleaved by the PV-encoded RdRp precursor (3CD) or protease (3C) protein. Studies have demonstrated that the viral proteinase 3CD cleaves PCBP2, thereby suppressing viral translation[24]. Although cleaved, PCBP2 retains two RNA-binding sites, allowing it to attach to the cloverleaf structure at the PV (+) RNA's 5′ untranslated region (UTR). This interaction is vital for PV RNA replication as it brings together the 3′ and 5′ ends of the viral RNA through interaction with another host protein family, the poly(A)-binding proteins[25].

In the context of tomato mosaic virus (ToMV) replication, two Arabidopsis thaliana membrane proteins, TOM1 and TOM3, are essential. They interact with the helicase-like ToMV replication protein 130K, facilitating ToMV replication [26]. Additionally, the SNARE-like protein, human vesicle-associated membrane protein-associated protein A (VAPA), serves as a membrane anchor for HCV replication proteins[27]. HCV replication proteins NS5A and NS5B66 interact with VAPA, a contact crucial for the association of NS5A and NS5B with intracellular ER-derived membranes, which serve as the site of HCV replication[28]. Moreover, LSM1 protein aids in RNA recruitment in Brome mosaic virus and HCV. In PV, HCV, and coronaviruses, host heterogeneous nuclear ribonucleoproteins promote RNA recruitment and (+) RNA and (-) RNA synthesis[16,20].



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Figure 1 Schematic representation of replication and transmission of positive-sense RNA viruses. RdRp: RNA-dependent RNA polymerase.

VIRAL-HOST INTERACTIONS AND IMMUNE RESPONSE

During a viral infection, the host's pattern recognition receptors (PRRs) detect viral pathogen-associated molecular patterns (PAMPs). These PAMPs represent distinct molecular attributes found in viruses that are absent in the host cell, enabling cells to differentiate between self and non-self entities, thereby initiating an immune response against infection [29,30]. The activation of these PRRs initiates intracellular signaling cascades involving adaptor proteins like MAVS and STING[6]. Subsequently, these adaptor proteins trigger the activation of kinases and transcription factors, which, in turn, promote the transcriptional upregulation of type I and type III interferons (IFNs) and the synthesis of antiviral proteins [31].

Efforts have been made to understand how virus infections affect host cell protein synthesis, but inhibiting host protein synthesis is not always necessary for successful virus replication[32]. Many viruses like paramyxoviruses, papovaviruses, and retroviruses typically do not block host protein synthesis during their replication. Moreover, mutant viruses that cannot effectively halt host protein synthesis are not necessarily impaired in their ability to replicate[13]. For instance, in the case of VSV mutants selected during persistent infections, they may have a reduced capacity to interfere with the host's translational processes, yet they can achieve higher virus titers during a lytic growth cycle compared to the wild-type virus[33,34]. In many instances, this inhibition is accompanied by an overall reduction in the rate of protein synthesis within the infected cell. This broad inhibition likely occurs at the initiation stage of protein synthesis, as observed by a decrease in the average size of polysomes in infected cells where examined[35].

Before the initiation of targeted cellular or humoral immune responses against a specific virus, the activation of apoptosis can serve as an initial defensive mechanism within host cells. This process aids in the removal of cells that have been infected by the virus, thereby restricting viral replication[36]. Molluscum contagiosum virus, equine herpesvirus 2, bovine herpesvirus 4, human herpesvirus 8, and herpesvirus saimiri encode FLICE-inhibitory proteins (FLIPs) that exhibit structural similarities to FLICE (caspase-8)[37]. These FLIPs engage with the Fas-associated death domain protein adapter within the host cell[20].

Studies on candidate genes and genome-wide associations have yielded important information on the genetic foundations of many infectious illnesses. The loss-of-function mutation known as CCR5Δ32, which results in the absence of CCR5 expression on the surface of host cells, confers resistance to HIV infection in homozygous individuals[31]. Polymorphic variations in HLA genes have been associated with a wide range of infectious diseases, including RNA viruses such as SARS, influenza, HIV, hepatitis C, rabies, West Nile fever, rubella, mumps, and measles, among others. Genetic association studies of this nature are pivotal for pinpointing HLA alleles that might be correlated with immune responses offering protection. Variations in HLA alleles have prompted inquiries into their potential role in the distinct immune responses observed between mild and severe coronavirus disease 2019 (COVID-19) cases, such as delayed immunoglobulin M (IgM) responses and elevated S protein immunoglobulin G titers in non-intensive care unit patients [38,39].

ANTIVIRAL DRUGS

RNA virus-mediated infections include HCV, SARS, influenza, Ebola, polio, measles, HIV, HTLV-1, Respiratory syncytial virus (RSV), and others. Most RNA viruses have single-stranded or double-stranded RNA as their genetic material. Viruses primarily possess RNA-dependent RNA polymerase for genome replication or have reverse transcriptase for genome replication. Idoxuridine was the first antiviral drug approved in 1963. Since then, 90 antiviral drugs categorized into 13 functional groups have been approved for the treatment of 9 infectious diseases. Antiviral drugs approved for RNA virus-mediated infections include trifluridine, vidarabine, entecavir, zidovudine, didanosine, lamivudine, abacavir, nevirapine, efavirenz, rilpivirine, ritonavir, indinavir, lopinavir, simeprevir, paritaprevir, raltegravir, elvitegravir, palivizumab, tenofovir disoproxil fumarate, sofosbuvir with ribavirin, amantadine, zanamivir, rimantadine, laninamivir octanoate, and favipiravir[40].

In the realm of influenza treatment, antiviral drugs primarily include adamantanes (M2 ion channel blockers) which can block an ion channel formed of M2 protein encoded by the M gene in influenza A virus. This category comprises two classes of drugs: Amantadine and rimantadine. Additionally, neuraminidase inhibitors, approved for use against both influenza A and influenza B viruses, include major drugs such as zanamivir, oseltamivir, Peramivir, and Laninamivir [41]. Another class, the RNA-Dependent RNA Polymerase Inhibitors, includes favipiravir, which hampers viral RNA synthesis. Notably, the absence of favipiravir-resistant viruses is a remarkable property, indicating its effectiveness as an antiviral medication. A novel class, the Polymerase Acidic Endonuclease Inhibitor, encompasses baloxavir marboxil, which inhibits the cap-dependent endonuclease of the viral RdRp complex. This disruption restricts mRNA production and prevents subsequent viral protein synthesis. Baloxavir marboxil is one of the most recently developed anti-influenza drugs[42].

Challenges in developing anti-influenza drugs stem from the virus's antigenic evolution mechanisms: Antigenic shifts and drifts in surface glycoproteins. Immunization against these processes is challenging due to their natural immunity combat mechanisms. Major limitations in antiviral treatment include antiviral resistance of most human influenza A virus strains to M2 inhibitors and the need for in vivo disease models for influenza-related research. Evaluating antiviral treatment efficacy poses challenges from the standpoint of drug resistance. One of the main hurdles in treating influenza is the emergence of drug-resistant influenza viruses due to current antiviral regimens. Alternative treatments include drug combination therapies that synergistically minimize drug resistance and reduce drug toxicity. Commonly used antiviral combination therapies include Oseltamivir + zanamivir (targeting the same viral protein), Baloxavir + favipiravir (targeting Cap-Dependent Endonuclease & RNA-Dependent RNA Polymerase), Baloxavir + oseltamivir (targeting Cap-Dependent Endonuclease & Neuraminidase), and Oseltamivir + amantadine + ribavirin (Triple-combination antiviral drug treatment targeting M2 Ion Channel, Neuraminidase & RNA-Dependent RNA Polymerase) [43]. Additionally, next-generation influenza virus inhibitor candidates in early-stage development include EIDD-2801 and Pimodivir, a cyclohexyl carboxylic acid analogue targeting the polymerase PB2 subunit to hinder influenza virus replication[44].

Moving to HCV treatment, initial antiviral efforts utilized IFNα monotherapy followed by ribavirin (RBV), a synthetic triazole guanosine analogue active against both DNA and RNA viruses. Pegylated IFN, a modified interferon with a prolonged pharmacokinetic profile, showed favorable results. Subsequently, direct-acting antivirals (DAAs) emerged to directly interfere with viral proteins, marking a significant breakthrough. Protease inhibitors (PIs) like boceprevir and telaprevir were among the first DAAs, substantially increasing sustained virologic response (SVR) rates. However, these drugs accelerated the generation of resistance-associated substitutions, leading to virological breakthroughs in almost all treated individuals. Compared to the prior standard regimen of Peg-IFN–RBV, Peg-IFN–RBV–triple therapy raised SVR rates in treatment-naive patients by about 30%[45].

The COVID-19 pandemic spurred progress in antiviral medication development, introducing both DAAs and host-based antivirals. Commonly used DAAs include remdesivir and molnupiravir, inhibiting RNA replication through interaction with RdRp. Nirmatrelvir targets the main protease or 3-chymotrypsin-like protease, while favipiravir and ritonavir are used for mild to moderate COVID-19 cases[46,47]. However, these drugs have limitations. Molnupiravir, in particular, has mutagenic potential for both the virus and the host. Host-based antiviral drugs like camostat and ivermectin target transmembrane protease serine 2. Other options include fluvoxamine, thapsigargin, and plitidepsin[48].

For HIV, various antiretroviral drugs have been developed, including Non-nucleoside reverse transcriptase inhibitors, Nucleoside reverse transcriptase inhibitors, PIs, Fusion inhibitors, CCR5 antagonists, Integrase strand transfer inhibitors, and Post-attachment inhibitors. Combination antiretroviral therapy or highly active antiretroviral therapy (HAART) uses these drugs in combination. Food and Drug Administration (FDA)-approved HAART treatments include bictegravir, emtricitabine, and tenofovir alafenamide, cabotegravir, and rilpivirine, doravirine, lamivudine, and tenofovir disoproxil fumarate, among others. Direct-Acting Anti-HIV Agents such as cabotegravir, doravirine, and islatravir, along with host-based antivirals like the monoclonal antibody ibalizumab, are being used or are in the developmental stage[47,49]. Despite their efficacy, these drugs pose various risks and side effects, including elevated liver enzyme levels, gastrointestinal toxicity, rashes, benign hyperbilirubinemia, nausea, headache, anemia, leukopenia, reversible peripheral neuropathy, lactic acid elevation, low phosphate levels, and CNS toxicity[50].

In the fight against polio, large-scale oral vaccination programs have been implemented through the Global Polio Eradication Initiative. However, no specific antipicornavirus drugs have been FDA approved. Promising candidates include pirodavir (capsid binders), rupintrivir (protease inhibitors), Enviroxime (protein 3A inhibitors), ribavirin (nucleoside analogues), and compounds like MDL-860, discovered as a broad-spectrum inhibitor of viruses, although its exact mechanism of action remains unknown[51,52].

Ebola virus, a highly lethal pathogen, lacks FDA-approved drugs or vaccines. However, several potential drugs are under investigation, including favipiravir, amiodarone, amodiaquine, chloroquine, clomiphene, toremifene, Brincidofovir, sertraline, and BCX4430[53].

RSV is a negative-sense, ssRNA virus that primarily causes acute lower respiratory tract infections in infants, children, adults, and immunocompromised individuals. Currently, the FDA has approved two drugs for RSV treatment: ribavirin (a guanosine analogue) and palivizumab (a monoclonal antibody)[54]. Several antiviral candidates against RSV are under clinical research and trial stages, including REGN2222, MEDI8897, and Motavizumab. Additionally, various fusion inhibitors, nucleoprotein inhibitors, nucleoside analogues, and non-nucleoside inhibitors are under development for RSV therapy[54].

HTLV-1 is an RNA virus mainly responsible for HTLV-1-associated diseases such as Adult T-cell leukemia (ATLL) and neurological disorders like HTLV-1 Associated Myelopathy (HAM). It is a retrovirus that affects CD4+ cells and, to some extent, CD8+ cells and dendritic cells. The treatment approach for ATLL diseases mainly involves a combination of drugs such as interferon α and zidovudine (IFN-α/AZT). However, there is no established treatment approach for HAM disorder[55]. These are some of the most commonly encountered RNA virus infections and the commonly used antiviral agents/drugs for their treatment.

RNA-BASED THERAPEUTICS

RNA holds significant potential for therapeutic applications. Growing understanding and recent advancements in RNA studies have paved the way for various innovative RNA-based therapeutic approaches. Several RNA-based therapeutic methods are gaining popularity and receiving clinical approval for use. These approaches offer certain advantages over antiviral drugs, conventional protein targeting, and DNA-based medicines. The key advantage lies in targeting the RNA of the virus, providing a broader and more efficient target. Small molecular drugs, in contrast, target about 0.05% of the human genome. Moreover, many targets of disease lack clearly defined active regions for binding of small molecule.

RNA-based treatments face significant challenges in terms of intracellular trafficking and metabolic stability. However, researchers have explored a variety of strategies to overcome these obstacles[56].

TYPES OF RNA-BASED THERAPEUTICS

RNA interference

RNA interference (RNAi) is an *in-vivo* cellular process which leads to silencing of RNA expression by using ds RNAs, this provides an intrinsic defensive mechanism against invading viruses and transposable elements. miRNAs and siRNAs are small oligonucleotide sequences of 20-22 nucleotides with definite structures composed of 5′-phosphate and 3′-hydroxyl endings and two 3′-overhang ribonucleotides on each duplex strand. Within the RNA-induced silencing complex, the endoribonuclease Dicer isolates the guide and passenger strands by cutting dsRNAs. While the passenger siRNA strand is broken down by the argonaute2 (AGO2) protein, the guide siRNA strand attaches itself directly to the target RNA and initiates AGO2-mediated cleavage[57]. When siRNAs bind to the promoter regions, they can also cause chromatin remodelling and histone changes in the nucleus, which silences transcription in addition to destroying cytoplasmic RNAs. This therapeutic approach has a lot of potential to be use in HIV, Influenza, SARS-CoV treatment[58].

Antisense oligonucleotides

In antisense oligonucleotides (ASOs)-mediated gene regulation, short single-stranded oligonucleotides (12-24 nt) are utilized. These oligonucleotides are complementary to specific RNA sequences through Watson-Crick base pairing, enabling them to alter the expression of proteins, reduce, or restore their expression[59]. There are two types of mechanisms used to modify expression: One is Occupancy-Mediated Degradation: In this mechanism, ASOs induce target mRNA cleavage by RNase H1 or ribozymes. The ASOs lead to the degradation of the target RNA, and 2nd is Occupancy-Only Mechanism in this mechanism, the target RNA is not directly degraded. Instead, various mechanisms are employed to modify expression. These include altering RNA splicing using splice switching, blocking miRNA binding to the target RNA, inhibiting or activating translation, and triggering nonsense-mediated mRNA decay[60].

CRISPR-based genome editing

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a prokaryotic defense system widely applied in genome editing. The CRISPR-associated protein (Cas) system enables precise genome sequence editing in mammalian cells and organisms, leading to the target gene's irreversible knockout or knockin. This system relies on guide RNA and Cas nucleases. The complex recognizes the protospacer adjacent motif sequence in the target RNA, initiating its activity. For effective genome editing, the Cas nuclease cleaves either the double-stranded DNA or a single-stranded RNA at the designated spot[61]. The most commonly used Cas systems are Cas9 and Cas13. The Cas9 system can target both double-stranded DNA and single-stranded RNA. This technique finds applications in detecting SARS-CoV-2, with CRISPR-Cas13-based assay designs used for detecting 67 diseases, including SARS-CoV-2, Zika virus, and dengue fever, among others. The CRISPR/Cas9 system aids in studying the regulation pathway of the influenza virus, representing an emerging field in developing antiviral therapies against diseases like HIV, Hepatitis, and SARS[62].

Aptamer: It is synthetically designed chemical antibodies, are single-stranded oligonucleotide sequences that specifically bind to and inhibit protein expressions. Aptamer selection is based on the methodical evolution of ligands by exponential enrichment. Aptamers function primarily by interfering with interactions between disease-related targets, such as those between proteins or between receptors and ligands[63]. Aptamers can also deliver therapeutic agents to specific cells. They have applications in controlling SAR-CoV-2 infection; slow off-rate modified aptamers (SOMAmers) are DNA-based aptamers that bind to specific S protein fragments of SARS-CoV-2, preventing virus interaction with ACE-2 receptors[64]. Aptamers like anti-CCR5 are designed to prevent the interaction of HIV with the T-cell GPCR receptor. They serve as prospective anti-HIV/AIDS drugs, offering targeted delivery of various therapeutic options through CCR5-targeted aptamers and aptamer-siRNA conjugates[65]. Aptamers are also used in viral disease diagnosis; aptasensors, electrochemical diagnostic tools, demonstrate advantages such as low cost, specificity and early detection for influenza A and HA glycoprotein virus particles[66].

mRNA-based therapeutics

mRNA is generally single-stranded and is transcribed from the antisense strand of DNA, carrying information about the expression of functional proteins. mRNA-based therapeutics represent the future of treating various refractory diseases, including infectious diseases, metabolic genetic disorders, cancer, cardiovascular diseases, and others. In this therapy, exogenous mRNA is introduced with the help of a carrier, acting as a vaccine or therapeutic agent that expresses the necessary functional protein. This approach offers several advantages over conventional therapies, including higher efficiency, faster design and production, adaptability, and lower costs. These benefits are possible due to a planned manufacturing method developed for in vitro transcribed mRNA[67].

However, there are challenges associated with mRNA-based therapies. The anionic nature of cell membranes makes it difficult for mRNA to translate functional proteins in the cytoplasm. Additionally, mRNA has a median intracellular half-life of approximately 7 hours, and efficient carriers are crucial for overcoming cellular barriers, improving immunogenicity, and addressing stability issues. Moreover, mRNA may trigger immunological reactions and related toxicities, hindering the development of mRNA-based treatments[68,69].

The basic steps for designing and manufacturing mRNA-based therapeutic agents include mRNA design and synthesis, mRNA entrapment, pharmacodynamics and safety evaluation, manufacturing, and clinical trials[70]. Quality control measures are essential, such as codon optimization for mRNA encoding the antigen, ensuring mRNA sequence identity and integrity, assessing nucleic acid quantity, 5′ capping, poly A tail length, optimizing 5′-UTRs and 3′-UTRs, and ensuring mRNA purity. During the drug delivery process, mass spectrometry analysis, nuclear magnetic resonance analysis, evaluation of lipid electric charges and ratios, assessment of lipid impurities, and transfection efficiency are crucial. For mRNA-lipid nanoparticle drugs, encapsulation efficiency, particle size, storage conditions, and zeta potential must be carefully considered[67,70].

Apart from the conventional linear mRNA form, there are other structural forms such as self-amplifying RNA derived from alphaviruses, circular RNAs, noncoding RNAs, and competitive endogenous RNAs. These diverse forms of mRNA can be utilized for therapeutics[71,72]. Correct delivery of mRNA inside living systems is pivotal, and various delivery systems like lipid nanoparticles (LNP), polymeric nanoparticles, cationic nanoemulsions, protamine-condensed mRNA, exosomes, extracellular vesicles, and mesoporous silica are used[73]. These delivery systems utilize electrostatic interactions, hydrogen bonds, or coordination interactions through methods like thin-film hydration, nanoprecipitation, or microfluidic mixing. Nanoparticle-based delivery systems enhance cell uptake, facilitate lysosomal escape, and accelerate translation, maximizing mRNA availability. Achieving effective in vivo distribution of mRNA necessitates tissue-targeted delivery of mRNA-based therapies[74]. Precision nanoparticle engineering has been developed to cross biological barriers, expanding its applications in various therapeutic areas for mRNA-based drug delivery[75].

LNPs are a popular mRNA-based delivery method targeting the liver. Current research focuses on improving LNP platforms for administration to additional tissues. Through the exact and predictable customization of LNPs to transport mRNA, Cas9 mRNA/single-guide RNA, and Cas9 ribonucleoprotein complexes to target organs *via* intravenous injection into the liver and lungs, selective organ targeting has emerged as a therapeutic method. Another critical aspect of drug delivery is the route of administration. While the majority of disorders can be treated with intravenous administration specific administration routes are tailored to the targeted organ or organ system[76,77].

Over the past three to four years, mRNA-based therapeutics have gained significant popularity due to their role in designing treatments for COVID-19 and are now extensively explored for their applications in other viral infections, specifically RNA virus infections such as influenza, SARS, HIV, HCV, RSV, and various cancer therapies. A growing number of well-funded biotechnology companies, including Moderna, CureVac, BioNTech, Argos Therapeutics, RaNA, Translate Bio, Ethris, Arcturus, and Acuitas, are investing billions of dollars in mRNA therapy. Clearly, one of the most compelling topics in medication research is mRNA, which is worth investigating in the long run[67].

MICRORNA (MIRNA) PATTERN AND ITS ROLE IN GLIOMAS

Circulating microRNAs, have gained significant attention in the field of cancer research, as potential non-invasive diagnostic biomarkers for various types of tumors, including gliomas (a type of brain tumor)[78]. miRNAs can be found in different body fluids, including blood (serum and plasma), and cerebrospinal fluid and can be transported between cells, including tumor cells and neighboring normal cells, through exosomes so these can be used as reliable biomarkers [79]. miRNAs interfere the protein translation through complementary base-pairing, or degrade mRNA, thus dysregu-

lation of miRNAs lead to tumor progression[80]. The miR-21 has the potential to predict the radiation necrosis compared to tumor progression[78]. The potential utility of miR-128 and miR-342-3p as biomarkers for assessing glioma grades and monitoring treatment response has been advocated[81]. Indeed, the profiling of these miRNAs can provide valuable insights into the presence, type, and stage of the tumour. Consequently, early detection and accurate diagnosis are crucial for patient prognosis and treatment planning, and survival.

For rapid and early detection of miRNAs high sensitive methods such as a toehold-mediated strand displacement reaction, the enzyme-free surface plasmon resonance imaging biosensing method, and the ultrasensitive electrochemical method, should be integrated with existing diagnostic modalities, such as imaging and molecular profiling (genetic and epigenetic markers), to enhance diagnostic accuracy and guide treatment decisions. But challenges are the heterogeneity nature of gliomas, the varying level of sensitivity and specificity of miRNA, small number of studies, lack of standardized protocols for miRNA isolation, quantification that makes miRNA to accurately detect all glioma types and stages[82]. With the advancement of glioma biology and miRNA function, possibility of miRNA-based tests may eventually become a valuable part of the diagnostic and screening toolkit for glioma patients.

DEVELOPMENT AND APPLICATION OF MRNA-BASED VACCINATION

Vaccination plays a important role in dealing with communicable diseases, remarkably contributing to global public health. In simple terms, vaccination aims to generate immunity against specific diseases by using vaccines. Conventional vaccine candidates mainly include whole organism vaccines (live attenuated or inactivated pathogens), subunit vaccines, viral vectors, *etc*., which have been crucial in disease prevention. However, the scalability, speed of development, and ability to respond to newly emerging pathogens of these conventional vaccination platforms are often limited.

Recently, mRNA-based vaccination has emerged as the most advanced technology offering various benefits. It provides a flexible framework for the quick and focused development of vaccines against infectious illnesses, such as viral outbreaks and new infections. mRNA vaccines also have the advantage of being developed and produced more quickly than traditional vaccinations, which frequently need expensive and time-consuming manufacturing procedures. When it comes to responding to emerging infections or developing variants, mRNA vaccines offer unparalleled flexibility and speed because they can be designed and manufactured in a few of weeks[83].

During recent times, mRNA-based vaccination against SARS-CoV-2 has been a groundbreaking discovery in tackling diseases. The design of mRNA vaccines is adaptable to various diseases by simply changing the mRNA sequence encoding the required antigen. However, their efficient delivery poses a substantial challenge due to their susceptibility to degradation, poor stability, and obstacles in reaching the targeted areas of action[84].

The delivery of mRNA-based vaccines is a major challenge due to the internal environment inside the cytoplasm and the need to pass through the cell membrane. Various delivery systems have been developed, among which nanoparticles have emerged as promising tools in mRNA vaccine delivery, overcoming the inherent limitations of naked mRNA molecules. These nanoscale delivery and protection systems offer effective cellular absorption, defense against enzymatic breakdown and controlled mRNA payload release. Furthermore, nanoparticles can be developed to increase balance, extend their duration of circulation, and enable targeted administration to immune cells or organs, enhancing the immunogenicity and effectiveness of mRNA vaccines[85].

Various types of nanoparticle-based carrier systems include LNPs, polymeric nanoparticles, peptides, and protamine-based delivery systems, as well as cationic nanoparticles. Among them, for the administration of mRNA vaccines, LNPs have become the most popular class of nanoparticles. Due to their hydrophobic core, LNPs can enclose and safeguard mRNA, facilitating effective cellular absorption and intracellular release. This strategy is highly effective in designing various mRNA vaccines against infectious diseases, including COVID-19[86,87]. LNPs are positioned as prospective tools for successful vaccination techniques due to their outstanding safety profiles, high transfection efficiency, and capacity to elicit robust immune responses. This nanoparticle-based delivery system for mRNA is also useful in addressing other issues like cancer immunotherapy, personalized medicine, and therapeutic interventions for genetic disorders[88].

TYPES AND MECHANISM OF ACTION OF MRNA VACCINES

Self-amplifying mRNA, trans-amplifying mRNA, and conventional mRNA are the three forms of mRNA vaccines that are now on the market. Conventional mRNA vaccines, also known as non-replicating or non-amplifying mRNA vaccines, mainly consist of untranslated regions (5′UTR, 3′UTR) and the coding part of mRNA, which by transcription produces one copy of the immunogenic protein[71]. Self-amplifying mRNA vaccines are genetically modified mRNA, incorporating engineered replicons from self-replicating RNA viruses. They possess 5′ and 3′ conserved sequence elements (CSE) that regulate viral RNA synthesis and facilitate attachment to viral or cellular proteins. Self-amplifying RNA contains non-structural proteins 1-4 (nsP 1-4) sequences[89]. Trans-amplifying mRNA is also genetically modified mRNA with 5′ and 3′ CSE. Trans-amplifying mRNA requires two RNA genes to be co-delivered: the mRNA without nsP 1-4 and the mRNA encoding nsP1-4 genes[90].

In mRNA vaccination technology, mRNA is synthesized outside the body, injected, and then transported across cell membranes for translation in the cytoplasm. Once in the cytoplasm, the mRNA is translated into the necessary protein by ribosomes. In the case of naturally occurring mRNA, this process occurs after the mRNA moves from the nucleus or cell membrane. The poly-A tail get attached to the poly-A-binding protein during translation, and the eIFs attach to the 5′UTR cap to start translation[70]. Ribosomes convert each codon, consisting of three nucleotides in the translated portion of the

mRNA, into an amino acid. After injection, immune cells internalize mRNA-LNPs, leading to the release of mRNA from the LNPs. Ribosomes recognize the mRNA, translating it into antigenic proteins. These proteins are broken down and processed by proteasomes, resulting in small peptides presented on the cell surface by major histocompatibility complex class I (MHC I) molecules, and activates CD8+ T lymphocytes to eliminate infected cells. These produced antigen can be broken down further by lysosomes, loading small peptides on major histocompatibility complex class II (MHC II) molecules, recognized by CD4+ T lymphocytes. These cells stimulate B cells to stimulate humoral immune responses and inflammatory cytokine release to stimulate cellular immunological responses. Successful mRNA translation into the necessary antigen requires recognition of the 5′ cap, poly-A tail, 5′UTR, 3′UTR, and translated region of the synthesized mRNA vaccine by the ribosomes[67].

Optimizing mRNA vaccines is essential for enhancing their stability, safety, and efficiency. Modifications in 5′ cap of the 5′UTR and also in the poly(A) tail of 3′UTR can regulate translational efficiency of mRNA. For instance, converting the mRNA cap into phosphorothioate when the mRNA vaccine is transfected in immature dendritic cells may increase stability and expression. Furthermore, by including altered nucleosides into the mRNA, the Toll-like receptor activation may be decreased or eliminated, improving the vaccine's safety *via* nucleoside modification[91].

DELIVERY SYSTEMS

The most commonly used mRNA vaccine delivery systems include LNPs, Polymeric nanoparticles, peptides, protein nanoparticles, protamine nanoparticles, and other systems like cationic lipid amphiphiles. Because they may effectively transfer mRNA intracellularly by merging with the lipid bilayer of early endosomes, lipid-based nanoparticles are frequently preferred. Through this procedure, the mRNA is shielded from RNase breakdown during systemic circulation and is allowed to enter the cytoplasm. These LNPs primarily has three important components, *i.e.*, 40%-50% ionizable lipids, 38%–45% cholesterol, and 10%–12% helper phospholipid. In certain cases, a fourth component, such as 1%-2% PEGylated lipid, is added. LNPs have been utilized for delivering mRNA vaccines and drugs against diseases like COVID-19 and influenza[92,93].

Polymeric nanoparticles involve the addition of low molecular weight polyethyleneimine with polyethylene glycol, which is then linked to cyclodextrin. Conjugation with cyclodextrin has been proven as a reliable and safe method for delivering mRNA. This approach has versatile applications and may lead to the development of specific antibodies[94].

Peptides and protein-based nanoparticles are extensively used due to their excellent biocompatibility and accessibility. Amphipathic peptides, with cationic or amphipathic amine groups (arginine), can facilitate delivery of mRNA into the cells. These peptides binds electrostatically to the mRNA, forming nano-complexes[95].

Protamine nanoparticles leverage the net positive charge of protamine, a cationic protein primarily composed of positively charged amino acids. Protamine can complex with nucleic acids, such as RNAs, enhancing their stability and shielding them from enzymatic degradation by nucleases. This property facilitates their delivery to specific tissues[96]. Protamine's cationic nature, attributed to an arginine-rich sequence, enables it to interact with negatively charged mRNA, making it valuable in the design of mRNA-based vaccines.

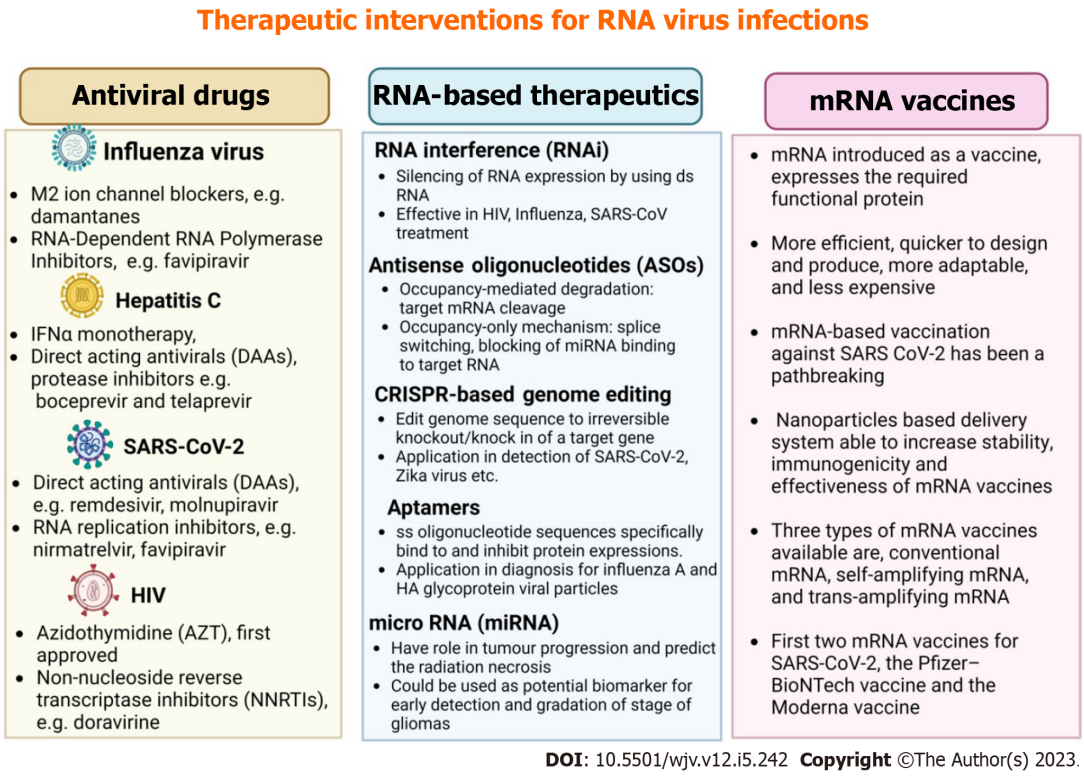
APPLICATION OF MRNA VACCINES

mRNA vaccines are primarily designed to generate immunity against infectious diseases and cancers. Due to the COVID-19 pandemic, these vaccines were developed more quickly, and as a result, the FDA approved the first two mRNA vaccines for SARS-CoV-2: The Moderna vaccine (mRNA-1273) and the Pfizer-BioNTech vaccine (BNT162b2)[97]. In addition to SARS virus mRNA vaccines, various mRNA vaccines for different viral infections such as influenza, HIV, Zika, and Rabies are under development. Numerous potential mRNA vaccine candidates are undergoing clinical trials, including mRNA-1345 against RSV (in phases two and three trials developed by Moderna), mRNA-1273 against SARS-CoV-2 variant B.1.351 (in phase two trials), and mRNA-1893 against Zika virus (in phase two trials). Apart from their application in preventing infectious diseases, mRNA vaccines also have broad applications in cancer treatment[98,99]. Figure 2 summarizes therapeutic interventions like antiviral drugs, RNA-based therapeutics, and mRNA vaccines for RNA virus infections.

FUTURE PERSPECTIVES AND CHALLENGES

The idea of personalized medicine may bring a great revolution in the field of medicine. Personalized medicine, an emerging practice of medicine uses a person's genetic profile to guide decisions which are made in regards to the prevention, diagnosis, and treatment of the disease[100]. The use of personalized medicine may be beneficial in many aspects, such as diagnostic accuracy improvement, better disease prevention, targeted therapy, reducing side effects, and the health care cost and promotion of research[101].

For instance, despite of rapid advancement in medical science, the modern medicine could not provide adequate treatment for COVID-19. In case of COVID-19 pandemic, it was established that observing the genetic background of each patient can contribute greatly to the drug effectiveness[102]. After the arrival of the challenges regarding treatment of coronavirus infection, the crucial roles of personalized medicine were realized by the physicians and healthcare workers.



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Figure 2 Summary of therapeutic interventions for RNA virus infections has been summarized. HIV: Human immunodeficiency virus; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; IFN: Interferon.

Hence, utilization of personalized medicine may stay as a potential therapeutic strategy for RNA virus mediated infections.

Previously, in late 2003, a coronavirus, *i.e.*, SARS-CoV-1 caused an outbreak of severe acute respiratory illness (now called SARS), and acute respiratory distress syndrome type findings[103]. In 2012, another coronavirus witnessed to cause Middle East respiratory syndrome, having a case-fatality rate of more than 35%. An outbreak of a disastrous pandemic occurred in late 2019, which in due course spread all over the world. Out of all these three coronavirus outbreaks the SARS-CoV-2 infection was newer and the first two outbreaks helped us to prepare for this third one[104].

The absence of suitable animal models, an insufficient understanding of the correlates of immune protection, and limited pharmaceutical industry investment are obstacles that affect the development of an HIV vaccine[105].

If the viral pathogens are zoonotic, it must be needed a prevention barrier to reduce their chances of first introduction to human population. 'One health' approach reduces the prevalence of viral pathogens with high zoonotic potential in animals and which in turn reduces the viral introduction into human population[106]. It is quite easy to monitor, treat or vaccinate the domestic animals, but is complicated for the wild animals. An inclusive surveillance plan must be executed for the wild animals in order to recognize the pathogens having possibility of transmission to human[107]. Advanced sequencing methods can be used for the surveillance to find out the variations which could have enhanced the zoonotic potential or pathogenicity[108]. Surveillance of bush meat market may be important for detecting such zoonotic viral pathogens.

For the management of any future pandemics, we must be ready with some preventable approaches. These approaches may be the non-pharmacological approach (such as mask wearing and social distancing), vaccine anticipation, and anticipating therapies to reduce morbidity and mortality[107].

Orthomyxoviruses, HCV, Ebola illness, SARS, influenza, polio, measles, and retroviruses including adult HTLV-1 and HIV are among the human diseases caused by RNA viruses. The genetic material of RNA viruses is RNA, which can be single-stranded or double-stranded[109]. Reverse transcriptase produces viral DNA that can be integrated into host DNA through its integrase activity, and viruses can also use RNA-dependent RNA polymerases to make copies of their genomes. Retroviruses, on the other hand, have two copies of their single-strand RNA genomes. Due to the error-prone nature of RNA-dependent RNA polymerases of RNA viruses, they live as quasispecies with several variants within their populations[110].

CONCLUSION

In this review article, we have highlighted the pathogenesis and recent advances in the treatment of RNA virus-mediated infections. We discussed RNAi and various RNA-based antiviral drugs, as well as the development of RNA vaccines and the challenges associated with their administration. Different types of vaccines exhibit distinct efficacy, and we

emphasized various strategies to enhance vaccine effectiveness. Additionally, our focus was on host-directed therapies, which represent an antiviral strategy. However, the development of these therapies poses significant challenges. Overcoming these challenges is crucial to transforming host-directed therapies into potent antiviral treatments.

FOOTNOTES

Author contributions: Mohanty P, Panda P, Acharya RK were involved in the data collection and validation; provided the first draft of the manuscript; Pande B, Verma HK and Bhaskar L prepared the figures and tables, wrote and finalized the manuscript; Pande B, Bhaskar L, and Verma HK designed the outline and coordinated the writing of the paper.

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MINIREVIEWS

Routine pediatric vaccinations during the COVID-19 pandemic: A review of the global impact

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic has put standard, routine childhood vaccinations at risk worldwide. The disruption in vaccine coverage has resulted in a negative impact on the health of children, with some races, ethnicities, age groups, areas of settlement, and parts of the world affected more than others. This literature review studied and examined the impact of COVID-19 on infant, child, and adolescent vaccinations. Retrospectively, the analysis showed a decline, delays, or interruptions in the coverage of vaccines during the pandemic and a decline in some countries' pre-pandemic and post-pandemic eras. Necessary attempts and efforts should be made for these delayed and missed vaccinations, as failure to do so could put children's health at risk. Thus, priority should be directed at instituting catch-up programs to support vaccine uptake and decrease the probability of acquiring vaccine-preventable diseases.

Key Words: Pediatric; Vaccination; Coronavirus disease 2019; Humans; Pandemics

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Core Tip: Studies worldwide have reported a decline in vaccination rates among the pediatric population because of the coronavirus disease 2019 pandemic. The disruption in vaccine coverage has resulted in a negative impact on the health of children, with some races, ethnicities, age groups, areas of settlement, and parts of the world affected more than others. Government efforts should be directed towards reversing these missed vaccinations.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic profoundly impacted the healthcare systems worldwide, resulting in significant and unintended consequences such as the disruption of routine health services, including immunization. A range of factors have contributed to these disruptions, such as travel restrictions, policies designed to reduce person-to-person contact, and concerns about viral exposure, resulting in the cancellation or postponement of patient visits. One result of these disruptions is a significant reduction in vaccine coverage, particularly in regions such as north Africa and the Middle East, south Asia, Latin America, and the Caribbean[1,2]. In the United States, data shows a decline in vaccination rates during the pandemic period, particularly during age-limited preventive care, and although vaccination rates rebounded during the expanded primary care period, they have yet to reach pre-pandemic levels[3]. To address this issue, China's Centers for Disease Control and Prevention (CDC) developed successful catch-up vaccination guidelines for children who missed or experienced delays in vaccination due to COVID-19[4]. Although sub-Saharan Africa experienced the lowest disruptions, data reveals a significant decline in vaccine coverage globally against vaccine-preventable diseases such as measles, diphtheria, tetanus, and whooping cough. Moreover, there are growing concerns on the heightened risk of outbreaks of other vaccine-preventable diseases such as polio[1]. Understanding the early impacts of the pandemic on vaccine coverage will help immunization programs determine how to continue to serve the health care needs of the population. Thus, this review aims to explore the impact of the COVID-19 pandemic on global immunization for other communicable diseases.

Methodology

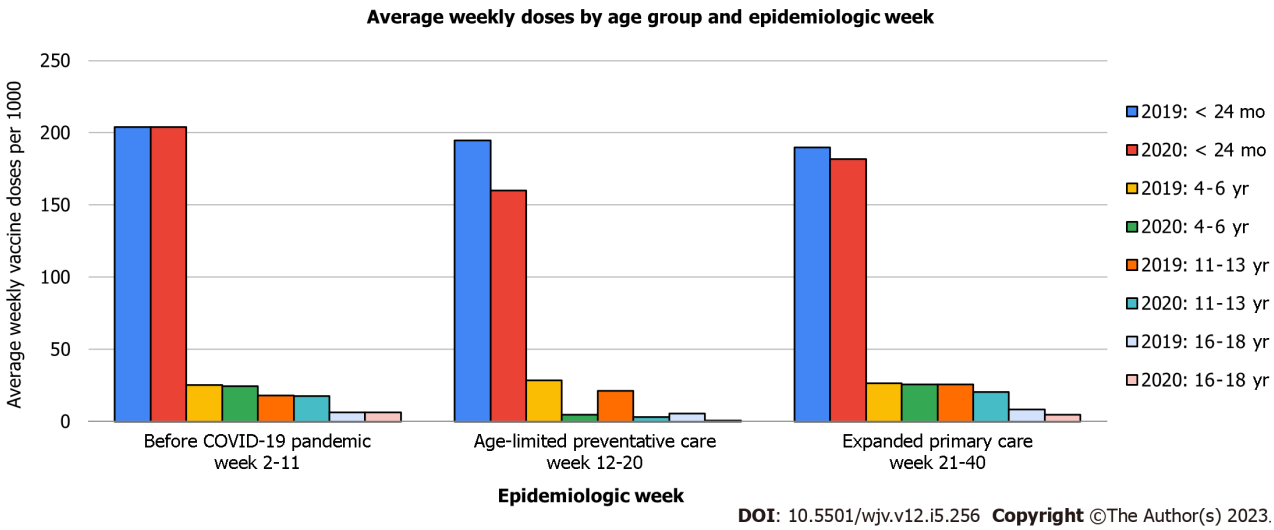
A mini-review was carried out to describe the epidemiological elements of the present global immunization decline in the pediatric population during the COVID-19 pandemic. An electronic literature review was conducted primarily using Google Scholar, MedLine Plus, and PubMed. The search for the assembled data was not limited to peer-reviewed studies published between December 2019 and September 2023. Grey literature sources were also visited to learn more about the decline in routine pediatric vaccinations during the pandemic. When selecting publications or manuscripts, keywords such as COVID-19 pandemic, immunizations of children, vaccinations, and disruption of vaccines were considered.

COVID-19 pandemic and routine childhood vaccination rates in the United States

Prior to the pandemic, the United States ranked highest coverage of most recommended pediatric vaccines due to its vaccination guidelines for documentation of vaccines for daycares and schools[3]. In September 2020, routine infant, child, and adolescent weekly vaccinations, showed that it was lower than 2019. Vaccination coverage had major distinctions between race and ethnicity across all ages and periods. The lowest was in African American children, and although disparities were present pre-pandemic, COVID-19 made these differences more significant, especially in the 18-mo-old age group[3]. When comparing the age groups and race for up-to-date vaccinations, the African American pediatric population was low on the spectrum. In the age group of 7 mo, the highest vaccine receiving population was in Asian infants (88.0%) and the lowest in African American infants (61.0%); in the 6-year-old age group, the highest was among Hispanic children (79.0%), and lowest was in African American children (70.0%); in the adolescent age group the lowest vaccinated were among African American adolescents (51.0%) and Caucasian adolescents (51.0%)[3]. Another disparity was seen in children living in rural areas, having increased missed vaccination doses, compared to their peers living in urban areas (33.3% *vs*. 15.2% unvaccinated children)[5]. These findings make it imperative to ensure that all children are targeted for their required vaccinations no matter their living situation.

Figure 1 compared the average weekly vaccine doses in 2019 and 2020 among those < 24 mo, 4-6 years, 11-13 years, and 16-18 years in the United States. Before the COVID-19 pandemic months: 2019 = 01/06/19-03/16/19; 2020 = 01/05/20-03/14/20; age-limited preventative care months: 2019 = 03/17/19-05/18/19; 2020 = 03/15/20-05/16/20; expanded primary care months: 2019 = 05/19/19-10/05/19; 2020 = 05/17/20-10/03/20. The vaccines administered in the study were: Children < 24 mo-hepatitis B (HepB); rotavirus; diphtheria, tetanus, pertussis (DTaP); *Haemophilus influenzae* type B (Hib) conjugate; measles, mumps, rubella (MMR); inactivated polio; varicella-zoster; and 13-valent pneumococcal conjugate. Children 4-6 years-MMR, varicella-zoster, and inactivated polio. Children 11-13 years-human papillomavirus (HPV), Tetanus toxoid, reduced diphtheria toxoid, acellular pertussis vaccine, and quadrivalent meningococcal conjugate. Adolescents 16-18 years-HPV and quadrivalent meningococcal conjugate[3].

The weekly vaccination rates shown in Figure 1, were measured by age group, period, and year. The pre-pandemic period in 2020 across all age groups was comparable to that in 2019. The period that saw a decline across all age groups was the age-limited preventative care in 2020 compared to 2019. The expanded primary care, was the only time that showed increased vaccination rates amongst all age groups. These findings further demonstrated how the COVID-19 pandemic affected the pediatric population regarding immunizations and how much this vulnerable population recovered from diseases.



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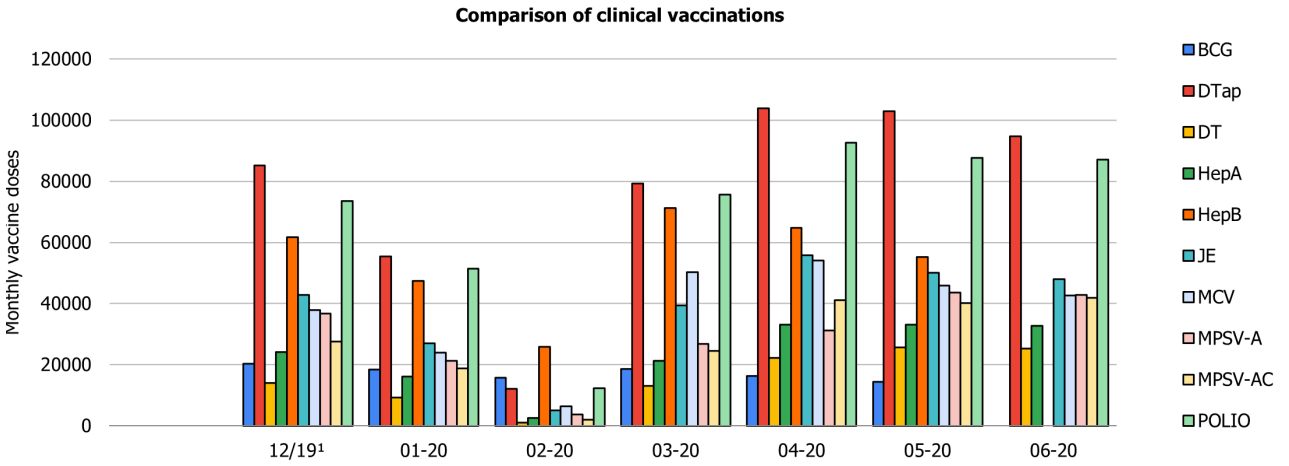
Figure 1 Average weekly vaccine doses by age groups and epidemiologic week[3].

Figures 2 and 3 compared the monthly vaccine doses administered in vaccination clinics in the United States from January 2020 to June 2020 with the baseline doses administered in December 2019[4]. Vaccinations compared are Bacillus Calmette-Guerin (BCG) vaccine, DTaP vaccine, diphtheria and tetanus toxoid vaccine, hepatitis A vaccine, HepB vaccine, Japanese encephalitis vaccine, measles-containing vaccine (MCV) vaccine, group A meningococcal polysaccharide vaccine, group A and C meningococcal polysaccharide vaccine, polio vaccine, and total vaccines. As depicted in Figure 2, weekly vaccination doses declined from the baseline in December 2019, especially in February 2020; however, most vaccines took a positive step in the catch-up phase of delivery doses in the following six months. One possible reason for the increase in required vaccinations administered to the pediatric population, beginning in late March 2020, could be the strategies executed to promote childhood vaccinations and reaching out to patients that were past due by the vaccine safety datalink, a collaboration with the CDC[6].

Discussion

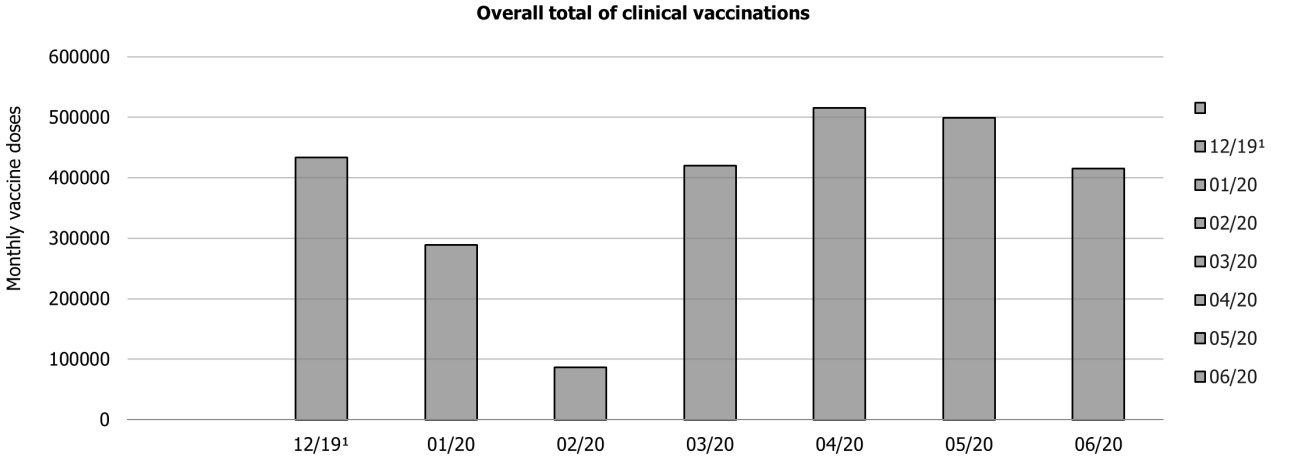
The usual immunization schedule was significantly impacted by the COVID-19 pandemic, particularly in the pediatric population. Even after taking COVID-19 precautions and returning to regular activities, the gaps in vaccine coverage raise the risk of vaccine-preventable infections. According to studies, the disruptions in normal immunizations caused by the pandemic provided risk of a 10.0% increase in mortality from diseases that can be prevented by vaccination[2]. Diphtheria-tetanus-pertussis, third dose vaccine (DTP3), and MCV first dose (MCV1) coverage were estimated to have fallen by more than 7.0% worldwide compared to expected coverage in the absence of COVID-19. More than 8 million additional children missed the DTP3 and MCV1 beyond expected estimates of vaccination gaps for 2020[2]. The global vaccination rate against tuberculosis (BCG vaccine), poliomyelitis vaccine (polio 3), and HepB third dose (HepB3) also dropped in 2020[7,8]. Over half of the African countries recorded a reduction of vaccination rates with Tanzania (polio 3) and Djibouti (DTP, Hep3, 3 doses of Hib vaccine (Hib3), 3 doses of pneumococcus conjugate vaccine (PCV3), MCV1, and MCV2) mainly impacted. In Asia, the COVID-19 pandemic caused a decline in pediatric vaccination coverage for almost all vaccinations. Larger decline (7.0%) of the BCG vaccination was observed in India. DTP3 has the highest reduction in Nepal (9.0%). The most pronounced decline observed for MCV1 occurred in Indonesia (12.0%) while the largest reduction in polio 3 vaccination was recorded in the Democratic People's Republic of Korea. Significant reductions in Europe occurred in Bulgaria, Ukraine, and Montenegro with MCV1, MCV2, BCG, and HepB3. Reductions in routine vaccination rates also occurred in the Americas with BCG, MCV1, PCV3, and polio3. In Canada, report of the Childhood National Immunization Coverage Survey collected in 2021, during the COVID-19 pandemic, showed that in-school vaccination programs for adolescents experienced delays and interruptions which differed across jurisdictions; however, by March 2021, national vaccination coverage rates were similar to the pre-pandemic level[9]. In Oceania, reductions were noted in some countries, notably in Samoa for MCV1 and Kiribati for MCV2[7,8]. Although the first half of COVID-19 was the most severe and caused the biggest impact, the second half showed promise with the increased number of vaccinations being administered; but despite this increase, millions of doses were still not being delivered. Some regions have been on the rise in their attempts to recover from the impact of COVID-19, but there are some areas that are still under the average for vaccinations in the pediatric population[2]. Regions such as sub-Saharan Africa were already below the global target before the pandemic, therefore recovery would still leave the pediatric population vulnerable to preventable diseases and affecting the long-term health of the children in that area[2]. Addressing the pre-pandemic gaps in coverage of missed childhood vaccinations needs to be considered as well as ensuring that all children receive their necessary immunizations; whether it was missed pre-pandemic or post-pandemic[5].

Moreover, those over six months of age are recommended to get the COVID-19 vaccine, according to the CDC, for the best defense[10]; despite that, COVID-19 typically causes minor illness, low hospitalizations, and infrequent post-acute consequences in young children[11,12]. However, there has been a correlate with COVID-19 infection in children and adolescents developing new-onset type 1 diabetes (T1D) mellitus and potentially other post-acute sequelae[13].



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Figure 2 Monthly vaccine doses of Bacillus Calmette-Guerin, diphtheria, tetanus, pertussis, diphtheria and tetanus toxoid, hepatitis A, hepatitis B, Japanese encephalitis, measles-containing, group A meningococcal polysaccharide, Group A and C meningococcal polysaccharide, and poliovirus vaccinations from December 2019 to June 2020[4]. [1]Marks the baseline when vaccine doses were administered in December 2019. BCG: Bacillus Calmette-Guerin; DTaP: Diphtheria, tetanus, pertussis; DT: Diphtheria and tetanus toxoid; HepA: Hepatitis A; HepB: Hepatitis B; JE: Japanese encephalitis; MCV: Measles-containing; MPSV-A: Group A meningococcal polysaccharide; MPSV-AC: Group A and C meningococcal polysaccharide; Polio: Poliovirus.



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Figure 3 Overall total vaccinations from December 2019 to June 2020[4]. [1]Marks the baseline when vaccine doses were administered in December 2019. Bacillus Calmette-Guerin vaccine doses administered were not available in June 2020. Hepatitis B vaccine doses administered were not available in June 2020.

Compared to pre-COVID-19 pandemic levels, the median glucose and hemoglobin A1C levels in newly diagnosed T1D children increased by 6.43% and 6.42%, respectively[14]. The pandemic of COVID-19 has increased the risk of juvenile new-onset T1D, diabetic ketoacidosis (DKA), and severe DKA worldwide[14]. During the early period of the B.1.1.529 (omicron) variant's predominance in the United States, the rate of COVID-19-associated hospitalization among children under five years of age peaked at 14.5 per 100000 in January 2022[11]. Roughly, this was five times the rate during the period of predominance of the B.1.617.2 (delta) variant in 2021[11]. It is worthy of note that 63.0% of infants and kids hospitalized in 2022 due to COVID-19 did not have any underlying medical issues[11]. It is of importance to encourage national pediatric associations to create national plans for integrating the COVID-19 vaccine into current immunization schedules, and according to the European Academy of Paediatrics campaign, "Vaccinate your child" should have support from national pediatric associations to reinstate postponed routine immunizations or vaccinate missed children[15].

Communities need to have a more inclusive system to provide opportunities for all children, no matter their living situation in order to receive their vaccinations. In 2019, it was found that coverage for the MCV1 vaccine which covers the first dose for measles was lower among children living in remote rural areas, compared to children living in urban areas [5]. Even a year after the COVID-19 pandemic started, challenges and gaps in the vaccines and immunizations system continued. Vaccinations systems are limited in tracking outside age groups with the current delay by notifying who and what vaccination is required for that given child. More strategic programming needs to be in place to reach the pediatric

population who missed vaccinations. Regions around the world can begin to develop stronger and healthier communities with a more up-to-date immunization requirement system, but if vaccines are still delayed, COVID-19 will continue to impact the children's health now and the future generations.

CONCLUSION

Studies worldwide have reported a decline in vaccination rates among the pediatric population because of the COVID-19 pandemic. Government efforts should be directed toward reversing these missed vaccinations. Strategies need to be strictly followed during times of crisis to prioritize the maintenance and reduced delays in routine childhood vaccination programs. Initiatives include low-income countries obtaining their missed vaccines through an organized programs such as increasing vaccine availability in remote areas, decreasing the wait times to care, as well as increasing public health awareness. To address these vaccine deficiencies and to maintain children's health protection from preventable communicable illnesses like polio and measles, public health procedures must be strictly followed. These infections can have negative consequences, including mortality, for younger children. Thus, stringent protocols should be safeguarded to avoid the increase in risk of other fatal illnesses, and emphasize the importance of providing consistency in maintaining childhood vaccinations to ensure the health and protection of preventable diseases in children.

FOOTNOTES

Author contributions: Locke J and Sanyaolu A did the conceptualization and methodology; Locke J, Marinkovic A, Hamdy K, and Balendra V did the writing-original draft preparation; Sanyaolu A did the writing-review, and editing of the study.

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Hepatitis E infection: A review

Humzah Iqbal, Bilal Fazal Mehmood, Aalam Sohal, Marina Roytman

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Abstract

Hepatitis E virus (HEV) is a small non-enveloped virus that is transmitted *via* the fecal-oral route. It is a highly common cause of acute hepatitis, particularly in low to middle income regions of Asia, Africa, and Central America. Most cases are self-limited, and symptomatic patients usually present with acute icteric hepatitis. A subset of patients including pregnant women, older men, those with pre-existing liver disease and immunocompromised patients however, may develop severe disease and hepatic failure. Immunocompromised patients are also at risk for chronic infection, and their immunosuppression should be decreased in order to facilitate viral clearance. HEV can also present with a variety of extra-intestinal manifestations including neurological, renal, hematological, and pancreatic derangements. The gold standard of diagnosis is HEV ribonucleic acid detection *via* nucleic acid amplification testing. Currently, there are no approved treatments for Hepatitis E, though ribavirin is the most commonly used agent to reduce viral load. Studies assessing the safety and efficacy of other antiviral agents for HEV are currently underway. HEV vaccination has been approved in China, and is currently being investigated in other regions as well. This review article aims to discuss the epidemiology, pathogenesis, presentation, diagnosis, complications, and treatment of Hepatitis E infection.

Key Words: Hepatitis E; Acute hepatitis; Chronic hepatitis; Viral hepatitis; Vaccination

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Core Tip: Hepatitis E is a common viral infection that has been increasing in developed nations. It usually causes a self-resolving acute hepatitis. It can sometimes lead to chronic hepatitis, and even cirrhosis/hepatic failure. Several subtypes exist, however the types responsible for infections in humans are generally spread *via* pork consumption or contaminated water. Treatment is usually supportive, however, ribavirin has shown efficacy in those with severe or chronic infection. Immunocompromised and pregnant patients should be evaluated with particular caution. Vaccination is currently licensed in China, and many studies are underway assessing vaccination efficacy in other nations as well.

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INTRODUCTION

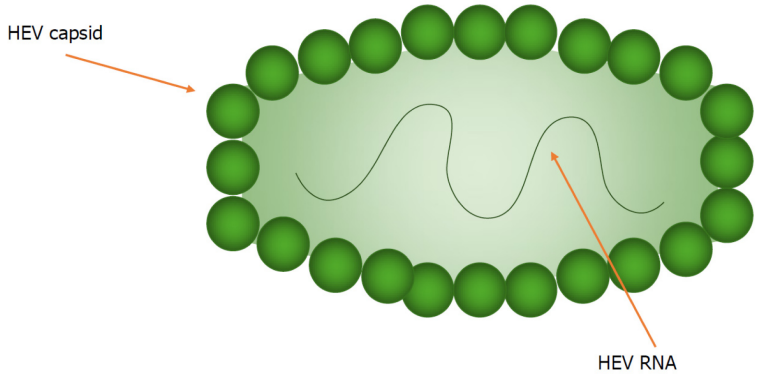
Hepatitis E virus (HEV) is a small non-enveloped virus in the *Hepeviridae* family. It was first discovered in the 1980s in a military camp in Afghanistan and was identified *via* electron microscopy in an individual who had symptoms of acute viral hepatitis[1]. Globally, HEV accounts for a significant proportion of liver disease, and is responsible for up to 70% of adult sporadic hepatitis cases in endemic regions. It is thought to be the most common etiology of acute viral hepatitis with an estimated incidence of 20 million cases yearly[2]. HEV is primarily transmitted *via* the fecal-oral route, and is responsible for multiple epidemics in developing countries within Asia and Africa[2]. However, it has become increasingly prevalent as a zoonotic viral infection in developed countries as well. Though HEV infection is self-limited in many cases, mortality rates and the incidence of fulminant hepatic failure (FHF) are significant in older male patients (6.5-10% mortality), pregnant patients (25%-30% mortality), and those with chronic liver disease (22%-43% mortality)[3]. Management is usually supportive, however, immunocompromised patients with chronic infection as well as high-risk populations may require antiviral treatment in order to prevent progression of liver disease and associated morbidity and mortality. HEV vaccination is currently approved in China, and multiple randomized control trials are underway in other endemic regions including Pakistan and Bangladesh.

EPIDEMIOLOGY

Hepatitis E is a hepatic infection caused by the HEV, a positive sense ribonucleic (RNA) virus, and is considered a global health issue. According to the World Health Organization, an estimated 20 million cases of HEV infection occur yearly resulting in 70000 deaths[4]. Particularly endemic to developing countries, HEV can be found in Asia, Africa, and Central America, and is especially prevalent in low to middle income regions of those areas[5]. The primary route in endemic areas of infection is fecal-oral, making areas with poor water sanitation particularly susceptible[6]. Four genotypes (1-4) are largely implicated in cases of HEV infection. In the above mentioned endemic regions, genotype 1 and 2 are predominantly the causative strains[5]. Sporadic cases and outbreaks can also occur, both in developed and under-developed regions, for which genotypes 3-4 are largely responsible and are most often secondary to zoonotic transmission, primarily from domestic pigs and wild boars[7]. Additionally, contaminated water can lead to viral transmission through shellfish, fruit, and salads[8,9]. In the United States (US), HEV is largely considered a travel-associated disease, usually brought into the country by travelers returning from endemic areas. However, a retrospective study of nationwide hospitalizations from 2010-2017 found that the incidence of HEV in the US has increased nearly two-fold[10]. In autochthonous (locally acquired) cases, HEV is thought to be predominantly caused by zoonotic transmission, usually originating from undercooked pork[4,7].

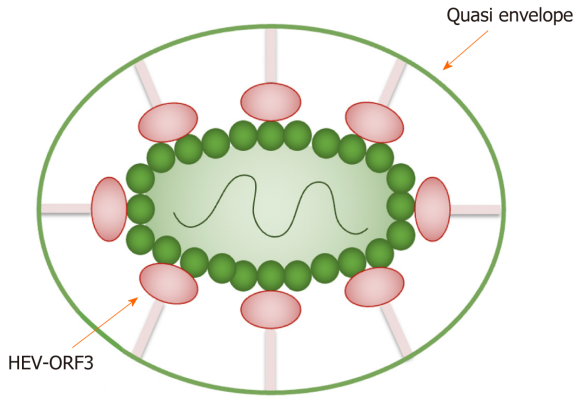
PATHOGENESIS

HEV is primarily spread fecal-orally *via* contaminated water or food (*e.g.* undercooked pork). The virus is a single-stranded, positive-sense RNA virus and is divided into two genera: Piscihepevirus and Orthohepevirus, the latter of which is divided further into 4 species (A-D). Interestingly, HEV-C is primarily spread by rats, and only shares 50%-60% identity with HEV-A. Some case reports describe HEV-C infection in transplant recipients, however, its infectious potential in humans remains unclear[11]. HEV-A has seven genotypes, of which 1, 2, 3, 4 and 7 infect humans[12]. HEV primarily targets hepatocytes however, until recently, the route of HEV reaching the hepatocytes was poorly understood. It is now thought that the virus first replicates enterically, with studies finding HEV RNA and ORF2 antigens in intestinal crypts of chronically infected patients. From here, the virus is thought to then enter the portal circulation and infect hepatocytes causing inflammation. The mechanism of viral entry into the hepatocyte is still poorly understood[13]. After entering the hepatocyte, the HEV genome is released into the cytoplasm where the virus hijacks intracellular machinery to replicate vital proteins and the RNA genome. ORF4 is critical for the replication process, and ORF3 is necessary for viral release from infected cells[12]. The HEV virion is shown in Figures 1 and 2.



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Figure 1 Naked hepatitis E virus virion structure. HEV: Hepatitis E virus.



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Figure 2 Quasi-enveloped hepatitis E virus virion structure. HEV: Hepatitis E virus.

The humoral immune response in conjunction with cellular immunity limits viral replication and allows the host to clear the infection, which is largely responsible for the self-limited nature of majority of cases[14]. In acutely infected patients, anti-HEV immunoglobulin M (IgM) antibodies peak in 6 wk, followed by anti-HEV IgG antibodies for long-term (years to decades) protection[13]. Acute infections are also associated with elevated T cells, with increases seen in both CD4+ and CD8+ populations, and subsequent release of pro and anti-inflammatory cytokines interferon (IFN) gamma and interleukin (IL)-10[15]. Further immune protection is provided by the innate lymphoid cell response, with natural killer (NK) cells combating viral infection with both cell-mediated cytotoxicity and by producing IFN gamma[12]. The same immune response responsible for limiting HEV infection is also largely the cause of hepatocellular damage and liver inflammation[12]. In their efforts to clear HEV from the host, CD8+ and NK cells along with the production of interferons, cause intrinsic damage to hepatocytes, leading to hepatitis[16].

CLINICAL PRESENTATION

Hepatitis E infection can present with a wide range of clinical manifestations. Most commonly, infected hosts remain asymptomatic. Symptomatic cases present as acute icteric hepatitis, which occurs in 5%-30% of infected hosts. This presentation includes a prodromal phase that lasts up to one week, manifested as fever, nausea, vomiting, and malaise [17]. Following the prodromal phase, dark urine and jaundice signal the onset of the icteric phase. During this time, mortality rates range from 0.5%-4%, however symptoms usually resolve spontaneously within a week[18]. More severe presentations can occur, such as fulminant HEV infection and/or progression to chronic HEV infection (sustained HEV replication for more than 3 mo). The populations most susceptible to these outcomes are pregnant and immunocompromised patients, such as solid organ transplant recipients and those with human immunodeficiency virus (HIV). Extra-hepatic complications can occur in both acute and chronic HEV infection, ranging from renal impairment to neurological symptoms (see section on complications). Extra-hepatic manifestations seem to be driven both directly by HEV replication and indirectly by immune system mediated effects[19].

DIAGNOSIS

Diagnosis of acute HEV infection initially involves the detection of anti-HEV antibodies (IgM). IgM antibodies appear in the acute phase of infection, and are detectable approximately 4 d after onset of jaundice. They may remain detectable for up to 5 mo. Anti-HEV IgG antibodies develop shortly after IgM, and remain in the serum for up to 14 years post-infection [20]. Sensitivity of traditional immunoassays range from 90-97%, with false positives up to 2.5%[21]. Immunocompromised patients may have delayed or absent seroconversion to anti-HEV antibodies, rendering this diagnostic modality insufficient in which case nucleic acid amplification testing (NAT) to detect HEV RNA from stool, serum, or liver biopsy can be used[21].

The gold standard test for confirming acute HEV hepatitis is detection of HEV RNA *via* NAT from serum, stool or on liver biopsy. However, there are several factors that make RNA detection a faulty method. Firstly, detection of HEV RNA is dependent on time of patient presentation. Following onset of illness, RNA is detectable in the serum up to 4 wk later, and up to 6 wk in the stool. Secondly, viral load can remain low and therefore even during the detectable periods, may not be captured by NAT[20]. The availability of HEV RNA testing in commercial labs remains limited, further restricting its use. Furthermore, the methodology of HEV NAT has not been standardized and large variability in sensitivities has been noted in the various techniques. Greater sensitivity has been noted in real-time reverse transcription polymerase chain reaction (RT-PCR) compared to nested RT-PCR assays[22]. A flowchart of HEV diagnosis is outlined in Figure 3.

COMPLICATIONS/SPECIAL POPULATIONS

Hepatic complications

Chronic HEV infection is primarily seen in immunocompromised hosts, and is exceedingly rare in immunocompetent patients. These patients largely consist of solid organ transplant patients, however other cohorts, such as HIV patients and chemotherapy patients, have also been described. These patients largely remain asymptomatic, and usually only have mild to moderate derangement in liver enzymes[23]. HEV-induced liver cirrhosis is a complication only seen in immunocompromised patients, often seen in HIV patients with low CD4 (< 200) counts or recent organ transplantation. Patients who have undergone solid organ transplant and are infected with HEV have a 50% chance of progressing to liver cirrhosis over several years, with 10% of patients reaching that point within 5 years[23-25].

Patients with pre-existing liver disease are at increased risk for severe HEV infection and liver failure, and should be evaluated with caution. A recent meta-analysis of 18 studies by Qiu *et al*[26] found a 35.8% rate of liver failure and 14.3% mortality rate in patients with chronic liver disease and superimposed HEV infection. Patients with cirrhosis had a two-fold increase in risk of liver failure and four-fold increase in risk of death compared to patients without cirrhosis. Similarly, a retrospective study by Tseng *et al*[27] demonstrated that HEV infection increases the rate of liver disease progression in patients with chronic hepatitis B (HBV) infection. The study also found an increased risk of mortality in patients in HBV-cirrhosis compared to non-cirrhotic patients (30% *vs* 0%, $P < 0.001$). Other studies have shown similar results regarding the effects of HEV superinfection in patients with pre-existing liver disease, prompting a discussion on vaccination for HEV in all patients with chronic liver disease in endemic regions (see section on vaccination)[28-30].

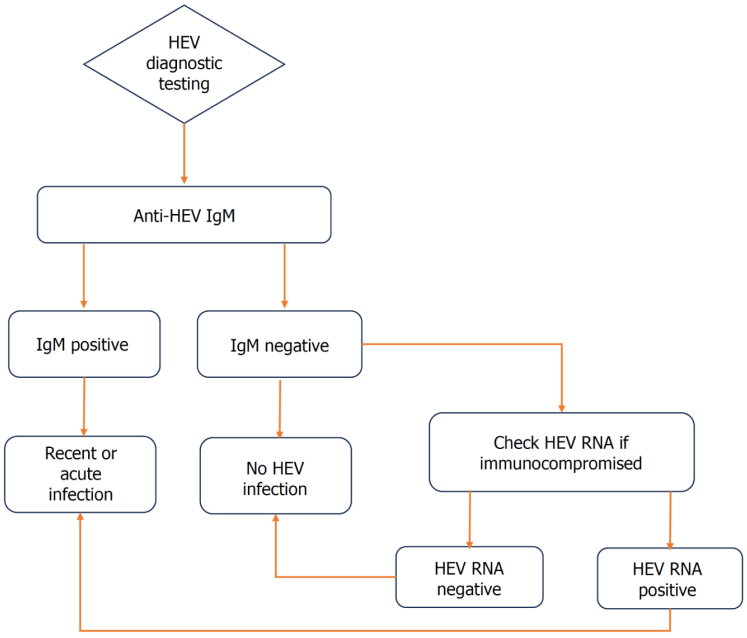
Pregnancy

HEV infection in pregnancy can be life-threatening with mortality rate up to 30% with HEV genotype 1 infection largely due to the development of HEV-induced FHF[12]. Studies have shown that pregnant patients with a progesterone receptor gene mutation, PROGINS, had reduced NK cell activity along with altered humoral and cellular immune responses[31]. Other studies have shown that pregnant patients have higher levels of tumor necrosis factor alpha, IL-6, and IFN-gamma, and that these cytokines had a significant positive correlation with HEV viral load, serum bilirubin, and prothrombin time. This raises the possibility that increased severity of HEV infection in pregnant patients may be mediated by increased levels of cytokines in the serum[12,32]. Non-host complications of HEV infection in pregnancy include vertical transmission of the disease, increased preterm births, stillbirths and neonatal mortality[33].

Extrahepatic manifestations

HEV infection can be complicated by extrahepatic manifestations ranging from neurological to renal complications. These manifestations can occur in both acute and chronic infection and are thought to arise from a combination of HEV replication in involved tissues and immune system related effects. Neurological pathologies have been widely reported and are seen largely in HEV genotypes 1 and 3. Reported disorders included Guillain-Barre syndrome, Bell's palsy, polyradiculopathy, neuralgic amyotrophy, acute transverse myelitis, and acute meningoencephalitis[18]. The pathophysiology behind neurological symptoms in HEV infection remains unclear, however it is hypothesized that the host immune response plays a large role, with studies showing that immunocompetent patients are more likely to have neurological complications than immunocompromised ones[18].

Renal injury is seen in both acute and chronic HEV infection, again with HEV 1 and 3 genotypes. Renal biopsies in affected patients show histological patterns of membranoproliferative glomerulonephritis and membranous glomerulonephritis. These complications are seen in both immunocompetent and immunocompromised patients. The pathophysiology is poorly understood, though it is possible that immune complex deposition, such as that seen in hepatitis C infection, could be the cause[18,34].



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Figure 3 Flowchart of diagnostic testing for acute hepatitis E virus infection. HEV: Hepatitis E virus; IgM: Immunoglobulin M.

Hematological complications such as aplastic anemia and thrombocytopenia have been reported, though the mechanism behind these complications is not well understood. Monoclonal gammopathy of unknown significance (MGUS), was found to have the prevalence of 26.2% according to a study by Woolson *et al*[35]. Studies have yet to determine whether the inflammatory state of HEV leads to increased prevalence of MGUS or if it is immunosuppression caused by MGUS that predisposes to HEV infection. Brown *et al*[36] found a significantly elevated risk of MGUS and multiple myeloma with infectious hepatitis (RR 1.82; 95%CI 1.25-2.65), though the study did not identify specific etiologies of the infectious hepatitis. Severe thrombocytopenia (platelet count < 20000) has been reported in rare cases of HEV infection, and as of 2010 there were only 6 known reported cases. Though the cause remains unclear, one theory is that the diminished platelet count is secondary to an immune-mediated response. This is supported by several of the patients having anti-platelet antibodies in their serum, response to immunosuppressive therapy, and increase in cell counts as the HEV infection resolved[37-39]. However, transient thrombocytopenia can be seen in a variety of inflammatory and infectious conditions, and further studies are needed to explore the underlying etiology given the rarity of this presentation.

Acute pancreatitis is another rare and poorly understood complication of HEV infection affecting only 6.2% of patients with acute HEV infection according to a study in Nepal[40]. Pancreatitis is more common, however, in patients with FHF, with one autopsy study finding pancreatitis in 44% of patients afflicted with FHF. It should be noted, however, that the study did not differentiate between different pathogens of viral hepatitis[41]. Interestingly, the few cases of HEV-related acute pancreatitis have almost exclusively been reported in India, where the virus is still endemic[42]. A single-center study in India by Raj *et al*[43] found that 2.1% of all patients with acute pancreatitis had acute HEV infection.

The pathophysiology of pancreatitis in HEV infection is poorly described in literature, though several theories have been postulated. One of the prevailing theories is that HEV virus is directly cytotoxic to pancreatic cells[44]. Other studies have hypothesized that swelling at the ampulla of Vater is caused by inflammation which inhibits secretion of pancreatic fluids[41]. One proposed cause is release of lysosomal enzymes from the liver which activate trypsin from trypsinogen and cause inflammation of the pancreas[40]. HEV-related pancreatitis is poorly understood, and further studies are warranted to elucidate the relationship between HEV infection and acute pancreatitis, as well as patient outcomes.

TREATMENT

Acute HEV infection usually does not require antiviral treatment, however, it should be considered in high risk patients or those with chronic infection. A small proportion of patients with acute HEV may progress to FHF or acute-on-chronic liver failure, particularly older men, pregnant women, and patients with underlying chronic liver disease. The most commonly used treatment in such cases is ribavirin, a guanosine analog[45]. Ribavirin has been shown to help clear the HEV virus and normalize liver enzymes[46,47]. The mechanism is not well understood, but is thought to deplete guanosine triphosphate pools, thus inhibiting HEV RNA replication. Though rare, ribavirin has been associated with hemolytic anemia, which can be severe in patients with underlying liver disease or chronic kidney disease[48].

Chronic HEV is most commonly seen in solid-organ transplant recipients, and the first step in management of these patients is to decrease the dose of immunosuppressive agents that target T-lymphocytes. Studies have shown that this step alone can lead to HEV clearance in 25%-33% of patients[49,50]. Pegylated interferon-alpha (PEG-IFN) should be avoided in patients with a history of solid organ transplants including heart, lung, pancreas, or kidney due to an increased risk of rejection. However, PEG-IFN can cautiously be used in patients with a history of liver transplantation, since the risk of rejection is lower[51,52]. Ribavirin is thought to be safe for use in the transplant population and therefore is the preferred agent. Retrospective studies have shown that 78-81% of patients with chronic HEV had undetectable HEV RNA in the serum 6 mo after completion of the ribavirin treatment course. This proportion increased to as high as 90% when treatment duration was prolonged in those who did not achieve sustained virologic response[53]. The regimen of choice is ribavirin 600 milligrams daily for 3 mo (unless longer course desired due to lack of sustained response). Similar to in hepatitis C (HCV) infection, some associations including the Grupo de Estudio de Hepatitis Virales (GeHEP) de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC) recommend a weight-adjusted dosing of ribavirin for treatment of HEV[54,55].

One of the limitations of ribavirin in the management of HEV is its potential for teratogenicity, given that pregnant patients are at increased risk of developing severe infection. However, a study by Sinclair *et al*[56] found no evidence for teratogenic effects in pregnant patients with HCV. The mortality rate of HEV infection in the third trimester of pregnancy is nearly 20%, so ribavirin should be considered in this population as organogenesis is generally complete prior to this phase of pregnancy. Additionally, severe hemolytic anemia is a potential complication of ribavirin, and patients should be monitored closely. Liver transplantation should be considered in patients that are progressing to liver failure despite appropriate management[56].

Sofosbuvir, the NS5B polymerase inhibitor used to treat HCV, has been a subject of investigation for the treatment of HEV. It has questionable efficacy as monotherapy for HEV, given the high relapse rates and incomplete initial response [57]. Studies have found mixed results regarding sofosbuvir/ribavirin combination therapy, with some showing efficacy in acute HEV infection and other showing inadequate response in solid-organ transplant patients with chronic infection [58-60]. Further studies and randomized clinical trials are needed to determine the proper treatment regimen and patient population best suited for these agents. A treatment flowchart for HEV infection is outlined in Figure 4.

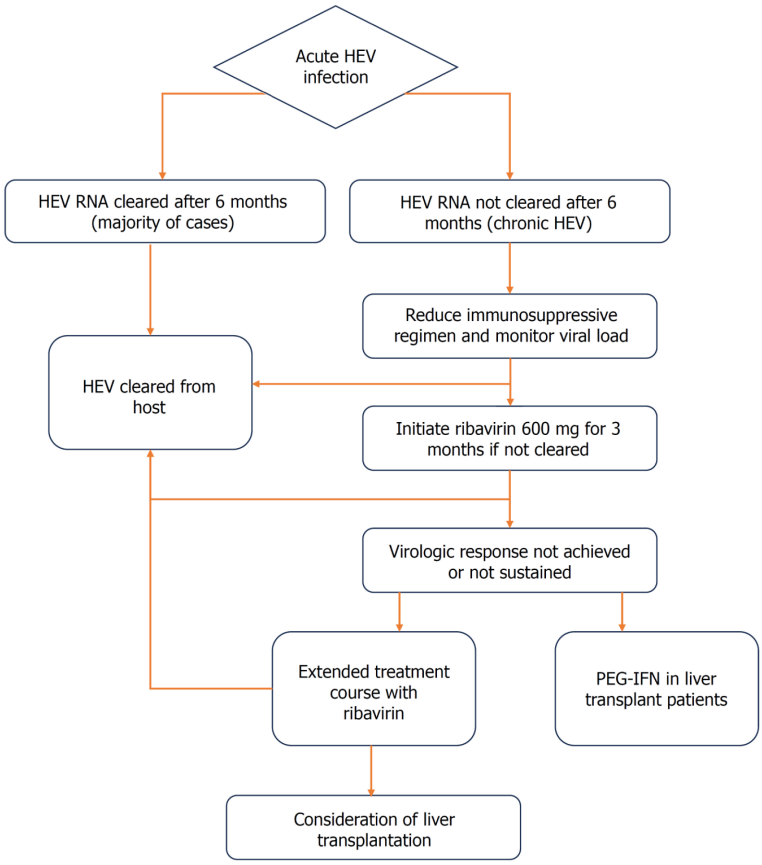
VACCINATION

There is only one currently approved vaccine for HEV, which was first licensed in China in 2011[61]. The HEV vaccine has been found to be effective in establishing long-lasting immunity against HEV genotypes 1 and 4[62,63]. In the study, 48420 healthy subjects received three doses (given at 0, 1 and 6 mo) of the vaccine and 48420 received placebo. No patients in the vaccine group developed HEV infection after 12 mo, compared to 15 patients in the placebo group, giving the vaccine 100% efficacy[64]. A clinical trial is currently recruiting in China to assess the long term effectiveness of the vaccine (NCT05976594)[65]. Li *et al*[66] found that the HEV vaccine is effective against genotype 3 in rabbit models, however its efficacy in humans remains unclear. Additionally, Sridhar *et al*[67] demonstrated that the HEV vaccine is not effective in HEV-C due to antigenic divergence, however, identified HEV-C1 p241 peptides as a potential vaccine candidate against HEV-C infection.

A topic of interest in recent years has been vaccination of at-risk populations. Ji *et al*[68] established a proof of concept demonstrating that administering the HEV vaccine to a German population with high levels of pork consumption would result in an 80% reduction in human HEV cases. Immunosuppressed patients (*i.e.* organ transplant recipients) are at increased risk for developing chronic infection, and therefore may warrant extra consideration for vaccination. However, rabbit models have demonstrated that HEV vaccination following initiation of immunosuppressive agents only conferred partial immunity, which did not improve with additional or increased vaccine doses[69]. Pregnant women are also considered high risk, and a randomized control trial is currently underway assessing the efficacy of HEV vaccination in pregnant women in rural Bangladesh[70]. A phase II randomized clinical trial assessing the safety and immunogenicity of the HEV vaccine in pregnant patients in Pakistan is expected to reach completion in 2025 (NCT05808166)[71]. Given the evidence showing worse outcomes and accelerated progression of liver damage in patients with pre-existing liver disease, HEV vaccination should be considered in these individuals. A major limitation of the HEV vaccine trials is the exclusion of patients with chronic liver disease, necessitating further studies to assess vaccine efficacy in this group[26].

CONCLUSION

HEV infection is a common cause of acute hepatitis worldwide that is usually characterized by an acute, self-limited course of symptoms including anorexia, nausea and jaundice. It has been the causative agent of many outbreaks in developing nations in Africa, Asia, and Central America, but has also been increasing in prevalence in developed countries. Risk factors such as pregnancy and chronic liver disease have been associated with a more severe disease course and immunosuppression with chronic HEV infection. Though there is currently no Food and Drug Administration approved treatment for HEV, ribavirin has shown efficacy in many studies and is the most commonly recommended treatment. The recombinant HEV vaccination licensed in China is the only vaccine currently available for HEV, and its long-term efficacy as well as its safety in various populations is being studied. Further studies are needed to establish a guideline-based treatment regimen for HEV in order to decrease global morbidity, mortality, and healthcare burden.



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Figure 4 Flowchart of treatment for hepatitis E virus infection.

FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Cohort Study

COVID-19 frequency and clinical course in children with asthma

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Abstract

BACKGROUND

The epidemic of severe acute respiratory syndrome coronavirus 2 infection, known as the coronavirus disease 2019 (COVID-19), has caused a global health concern. Since its emergence, numerous studies have focused on various clinical manifestations and outcomes in different populations. However, studies are ongoing as the consequences and impact of COVID-19 in children with chronic diseases such as asthma are controversial.

AIM

To fill this research gap by retrospectively evaluating the course, laboratory, and clinical findings of COVID-19 among 414 asthmatic children followed up from the pediatric allergy outpatient clinic and known to have had COVID-19.

METHODS

The data of 5510 patients over the age of 5 diagnosed with asthma in our hospital's data were retrospectively scanned with specific parameters using protocol numbers from the hospital filing system. The data included retrospective evaluation of pulmonary function test results before and after COVID-19, routine hematological and biochemical parameters, sensitization states (total IgE, specific IgE, and skin prick test results), and radiological (computed tomography) findings. To inquire about the course and symptoms of COVID-19, asthma patients or their parents were then called and evaluated with a questionnaire.

RESULTS

As a result of retrospectively scanning the data of 5510 asthma patients over the age of 5, it was determined that 414 (7.5%) patients had COVID-19. The mean age of 414 patients was 17.18 ± 4.08 (min: 6; max: 28) years. Two hundred and three of our 414 patients are male, and 211 are female. When their vaccination status was questioned, 21.5% were vaccinated. When the symptoms of our 290 patients were

questioned, it was stated that 59.0% had fever symptoms. The rate of using regular prophylactic asthma medications was 19%. The rate of using salbutamol in asthma was found to be 22%. The rate of patients using methylprednisolone was 1%. Emergency service admission was 17.2%, and hospitalization was found to be 4.8%. Leukopenia (< 4000) was found in 14.1% of patients, and 8.08% of our patients had neutropenia (< 1500). Lymphopenia (< 1500) was detected in 44.4% of patients, and lymphocytosis (> 4000) was found in 5.05% of patients. In 65% of our patients, the C-reactive protein value was elevated. A high aspartate aminotransferase and alanine aminotransferase value was detected in 3.2% and 5.4% of patients were found, respectively. 31% of patients had an elevated lactate dehydrogenase value. Typical radiological findings for COVID-19 were detected in 3/309 of patients.

CONCLUSION

According to our study, there is a correlation between the severity of COVID-19 and asthma symptoms and the course of the disease. However, it is worth noting that the retrospective nature of the study and the differences in sample size, age, and demographic characteristics between the two groups do not allow for an optimal comparison. Therefore, further investigation is needed to explore the relationship between COVID-19 and asthma, and it can be suggested that COVID-19 may trigger asthma attacks and asthma may impact the course of COVID-19.

Key Words: COVID-19; SARS-CoV-2, Children; Asthma; Exacerbation; Allergy

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Core Tip: In our comprehensive retrospective study, we have made a noteworthy observation indicating a correlation between the severity of coronavirus disease 2019 (COVID-19) and the presence of asthma symptoms, which also appear to influence the course of the disease. These findings offer valuable insights into the potential interaction between COVID-19 and asthma. Given the complexity of this relationship and its possible implications for patient management, further in-depth investigations are warranted to elucidate the precise mechanisms and associations at play, aiming to improve our understanding and management of both conditions.

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INTRODUCTION

The epidemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, known as the coronavirus disease 2019 (COVID-19), has caused a global health concern. Since its emergence, numerous studies have focused on various clinical manifestations and outcomes in different populations. However, studies are ongoing as the consequences and impact of COVID-19 in children with chronic diseases such as asthma are controversial.

Asthma, a chronic inflammatory disease of the airways, is one of the most common non-communicable chronic childhood diseases among children. According to the American Centers for Disease Control and Prevention's (CDC) data, approximately 6.5% of children under the age of 18 in the United States have asthma[1]. Globally, it is estimated that 262 million people have asthma in 2019[2]. Children with asthma are often more susceptible to viruses that infect the respiratory tract, including common coronaviruses[3]. However, the relationship between asthma and a novel coronavirus, SARS-CoV-2, in the pediatric population has not yet been fully established.

This study aims to fill this research gap by retrospectively evaluating the course, laboratory, and clinical findings of COVID-19 among 414 asthmatic children followed up from the pediatric allergy outpatient clinic and known to have had COVID-19. We hope to shed light on the clinical manifestations, severity, and prognosis of COVID-19 in this specific pediatric population, helping develop targeted treatment strategies.

MATERIALS AND METHODS

In our study, the data of 5510 patients over the age of 5 diagnosed with asthma in our hospital's data were retrospectively scanned with certain parameters using protocol numbers from the hospital filing system. This study's approval was obtained from the Sakarya University Faculty of Medicine clinical research ethics committee (Decision No: E-71522473-050.01.04-128344-122).

The data included a retrospective evaluation of pulmonary function test results before and after COVID-19, sensitization states (total IgE, specific IgE, and skin prick test results), and radiological (computed tomography) findings.

Also, routine hematological and biochemical parameters of the patients including hemoglobin, leukocyte, neutrophil, lymphocyte, eosinophil, platelet counts, C-reactive protein (CRP), sedimentation rate, urea, aspartate aminotransferase (AST)-alanine aminotransferase (ALT), prothrombin time (PT), activated partial thromboplastin time (aPTT), D-dimer, lactate dehydrogenase (LDH) and finally total IgE values were evaluated. These values were collected retrospectively from our hospital filing system and laboratory evaluations performed within 2 wk of having COVID-19.

In order to inquire about the course and symptoms of COVID-19, 414/5510 (7.5%) asthma patients who had COVID-19 or their parents were then called and evaluated with a questionnaire.

Statistical analysis

Descriptive analyses were performed to provide information on the general characteristics of the study population. The Kolmogorov-Smirnov test was used to evaluate whether the distributions of numerical variables were normal. Accordingly, one-way ANOVA or Kruskal Wallis test was used to compare the numeric variables among three groups (for multiple comparisons of ANOVA and Kruskal Wallis tests, Sheffe and Dunn's test was used). Eta squared was calculated for the effect size of ANOVA or Kruskal Wallis test. The numeric variables were presented as mean ± standard deviation. The Chi-Square test compared categorical variables. Cramer V coefficient was calculated for the effect size of the Chi-Square test. Categorical variables were presented as a count and percentage. A P value < 0.05 was considered significant. Analyses were performed using SPSS statistical software (IBM SPSS Statistics, Version 22.0. Armonk, NY: IBM Corp.)

RESULTS

As a result of retrospectively scanning the data of 5510 asthma patients over the age of 5, it was determined that 414 (7.5%) patients had COVID-19. When their intra-familial contamination status was questioned, 36% stated that they had positive intra-familial transmission cases; the rest were not intra-familial transmission. The intra-familial transmission was highest in atopic patients. When their vaccination status was questioned, 21.5% were vaccinated. The mean age of 414 patients was 17.18 ± 4.08 (min: 6; max: 28) years. Two hundred and three of our 414 patients are male, and 211 are female. Three hundred and nine of our 414 patients could be reached by phone.

When the symptoms of our available 290 patients were questioned, it was stated that 59.0% had fever symptoms. Interestingly, one of our patients stated that he had a decrease in fever and was even admitted to the emergency department with the risk of hypothermia. Fatigue 39.0%; muscle pain 33.8%; headache 33.2%; cough 32.5%; sore throat 23%; joint pain 23%; shortness of breath 18.8%; runny nose 10.0%; nausea 9%; vomiting 6.8%; diarrhea 5%; loss of taste and smell 5.02% patients were reported to have symptoms. There was no significant difference among the rest of asthma group (others), vaccinated and atopic asthma patient groups, except for shortness of breath (Table 1; Figure 1).

The rate of using regular preventive/prophylactic asthma medications was 19%. The rate of using salbutamol in asthma attacks was found to be 22%. The rate of patients using prednol (methylprednisolone) is 1%. Emergency service admission was 17.2%, and hospitalization was found to be 4%. The number of days hospitalized patients stay in the hospital varies between 3-10 d. In addition, it was determined that one of our patients was hospitalized in the intensive care unit. A table shows the evaluation of therapeutic features in different patient groups (Table 2; Figure 2).

Upon inquiry about the general medications employed by our patients during their COVID-19 illness, the investigation yielded the subsequent findings. Out of the participants, 126 individuals utilized antipyretic-analgesic group drugs, 22 antiviral drugs, 18 leukotriene receptor antagonists, and 11 were treated with antibiotic group drugs. Furthermore, six patients opted for 2nd generation antihistamine group drugs, and two patients utilized antiemetic derivative drugs. Among the sample of 272 patients, 173 individuals (63%) reported needing medication during the COVID-19 period.

After investigating the recovery times of our patients, the findings showed that out of 309 patients, 272 (88%) could heal within 1-15 d. Only a small percentage of patients, three (0.9%), required a longer recovery time of 30 days. Additionally, two patients needed more extensive recovery times of 45 and 150 d, respectively.

According to these parameters, the hemogram values of 97 of our patients could be reached (Table 3). Leukopenia was found in 14/96 (14.6%) patients, and leukocytosis was found in 12.5%. Eight point three percent of our patients had neutropenia (< 1500), and 12.5% of patients had neutrophilia (> 7500). Lymphopenia (< 1500) was detected in 45.4% of patients, and lymphocytosis (> 4000) was found in 5.2% of patients. In our 6.2% of patients, elevated eosinophil (> 500) values (eosinophilia) were detected. 6.2% of patients had thrombocytopenia (< 150000); 1.03% of patients had thrombocytosis (> 450.000) (Table 4; Figure 3).

From the parameters of the coagulation system, the number of patients whose PT values were reached is 45. A high PT value (> 13.2) was detected in 11.1% of patients. The number of patients whose aPTT value was reached is 42. aPTT values were elevated (> 33.5) in 4.7% of our patients. D-dimer value was elevated (> 500) in 11.7% of our patients (Table 4; Figure 4).

When we look at the other biochemical parameters, the CRP value of 84 of our patients was reached. In 65% of our patients, the CRP value was elevated. High sedimentation value was detected in 14% of our patients. The number of patients whose AST and ALT values were reached is 92. An increased AST and ALT value was detected in 3.2% and 5.4% of patients, respectively. The number of patients whose LDH value was reached was 67, and 31.3% had an elevated LDH value. Total IgE values of 52 patients were reached. A high (> 150) total IgE value was found in 38.4% of patients (Table 4; Figure 4).

Table 1 Evaluation of clinical features in different groups of asthma patients

	Others[1] (*n* = 169)	Vaccinated patients (*n* = 57)	Atopic patients (*n* = 64)	*P* value	ES
Gender (male)	128/169 (47.6)	26/57 (45.6)	38/64 (59.4)	0.199	0.091
Fever	100/163 (61.3)	27/57 (47.4)	40/63 (63.5)	0.130	0.120
Fatigue	60/163 (36.8)	27/57 (47.4)	24/63 (38.1)	0.365	0.084
Headache	51/163 (31.3)	23/57 (40.4)	22/63 (34.9)	0.453	0.075
Muscle pain	62/163 (38)	17/57 (29.8)	18/63 (28.6)	0.296	0.093
Cough	53/163 (32.5)	24/57 (42.1)	17/63 (27)	0.205	0.106
Sore throat	41/163 (25.2)	15/57 (26.3)	10/63 (15.9)	0.280	0.095
Joint pain	43/163 (26.4)	11/57 (19.3)	13/63 (20.6)	0.452	0.075
Shortness of breath	23/163 (14.1)	17/57 (29.8)	14/63 (22.2)	**0.026**	0.160
Runny nose	19/163 (11.7)	5/57 (8.8)	5/63 (7.9)	0.653	0.055
Nausea	15/163 (9.2)	5/57 (8.8)	6/63 (9.5)	0.990	0.008
Vomiting	10/163 (6.1)	4/57 (7)	6/63 (9.5)	0.660	0.053
Diarrhea	9/163 (5.5)	4/57 (7)	2/63 (3.2)	0.624	0.057
Loss of taste and smell	6/163 (3.7)	6/57 (10.5)	3/63 (4.8)	0.148	0.119
Intra-familial contamination	59/164 (36)	15/57 (26.3)	31/64 (48.4)	**0.039**	0.151

[1]This column includes asthma patients who are neither atopic nor vaccinated.
Statistics were shown as *n*/total *n* (%). ES: Effect size (Cramer v coefficient).

Table 2 Evaluation of therapeutic features in different groups of asthma patients

	Others[1] (*n* = 169)	Vaccinated patients (*n* = 57)	Atopic patients (*n* = 64)	*P* value	ES
Preventive/prophylactic asthma medication usage	31/164 (18.9)	9/57 (16.1)	16/62 (25.8)	0.372	0.084
Salbutamol usage	34/164 (20.7)	11/57 (19.6)	20/63 (31.7)	0.169	0.112
Prednol usage	2/164 (1.2)	1/57 (1.8)	0/64 (0)	0.619	0.059
Emergency service admission	27/164 (16.5)	8/57 (14)	14/64 (21.9)	0.485	0.071
Hospitalization	7/164 (4.3)	3/57 (5.3)	4/64 (6.3)	0.866	0.038

[1]This column includes asthma patients who are neither atopic nor vaccinated.
Statistics were shown as *n*/total *n* (%). ES: Effect size (Cramer v coefficient).

Computed tomography (CT) reports of our 7/309 patients that may be associated with COVID-19 have been obtained. When we evaluated our patients' very few CT findings regarding COVID-19, findings that were shown as typical findings for COVID-19[4] were detected in 3 of our patients. The findings detected in the remaining patients were classified as findings in other viral pneumonia types that are not specific to COVID-19.

COVID-19 in asthmatic patients with atopy

When we look at patients with COVID-19, it was determined that 110/414 (26%) patients had atopy/sensitivity. In the filing system, inhalant allergy was found in 100 patients, food allergy in 4 patients, and inhalant and food allergy in 6 patients.

Considering that 64 patients could be reached by phone; in 48.4% of patients, intra-familial transmission was detected. In 41.6% of patients, it was transmitted from outside the family. 27% of our patients were vaccinated before contracting COVID-19. A higher incidence of intra-familial transmission was detected in these patients compared to the others.

Of 64 patients, 63.5% reported fever as a symptom, while 38.1% experienced fatigue and 34% had headaches. Muscle pain was reported by 28% of the patients, while 27% had a cough. Joint pain and shortness of breath were reported by 20% and 22% of patients, respectively; while sore throat and runny nose were reported by 15% and 7%, respectively. Vomiting was reported by 9% of patients, while 9% experienced nausea and 3.2% had diarrhea. Additionally, 4% of patients experienced loss of taste and smell (Figure 1B).

Table 3 Evaluation of various laboratory parameters in different groups of asthma patients

	Others[4], (*n* = 169)		Vaccinated patients, (*n* = 57)		Atopic patients, (*n* = 64)		*P* value	ES
	n	mean ± SD	*n*	mean ± SD	*n*	mean ± SD		
Age	269	16.57 ± 4.16[a]	57	18.86 ± 3.43[b]	64	15.77 ± 3.87[a]	**< 0.001**[1,3]	0.049
COVID-19 age	269	14.71 ± 4.09[a]	57	17.28 ± 3.55[b]	64	13.83 ± 3.75[a]	**< 0.001**[1,3]	0.063
Hemoglobin	68	13.13 ± 1.44	12	13.66 ± 1.57	17	12.96 ± 1.83	0.456[1]	0.017
Leucocyte	67	6803.79 ± 2825.88	12	6162.5 ± 2173.43	17	7068.24 ± 4155.47	0.903[2]	0.007
Neutrophil	67	4119.30 ± 2601.78	12	3563.33 ± 1721.86	17	3762.94 ± 2089.5	0.920[2]	0.008
Lymphocyte	68	1861.94 ± 939.88	12	1924.17 ± 1008.59	17	2503.53 ± 2908.44	0.915[2]	0.027
Eosinophil	67	141.91 ± 171.81	12	157 ± 149.52	17	146.29 ± 190.62	0.327[2]	0.001
Platelet	68	245864.71 ± 74049.74	12	273750 ± 77324.73	17	232352.94 ± 66343.75	0.368[2]	0.024
CRP	62	10.28 ± 20.13[a]	8	7.84 ± 5.34[a]	14	2.85 ± 2.41[b]	**0.016**[2,3]	0.025
Sedimentation	5	11 ± 7.52	2	5 ± 1.41			-	-
AST	63	25.58 ± 11.28	12	22.92 ± 5.23	16	27.06 ± 12.68	0.829[2]	0.011
ALT	63	19.13 ± 13.72	12	20.17 ± 14.83	16	20.06 ± 17.47	0.908[2]	0.001
Urea	61	21.5 ± 6.2	12	21.35 ± 7.18	16	22.45 ± 6.81	0.727[2]	0.004
PT	33	12.07 ± 1.24	4	11.65 ± 0.95	8	12.49 ± 1.25	0.512[1]	0.031
aPTT	31	28.87 ± 3.4	4	28.53 ± 1.97	7	28.96 ± 2.17	0.974[1]	0.001
D-Dimer	48	508.98 ± 953.33	9	334.78 ± 206.13	11	265.18 ± 128.16	0.647[2]	0.015
LDH	47	243.44 ± 88.75	8	202.59 ± 54.89	12	225.16 ± 87.56	0.312[2]	0.027
Total IgE	37	176.05 ± 252.36[a]	2	174.5 ± 144.96[a,b]	13	1127.04 ± 1325.03[b]	**0.009**[2,3]	0.274

[1]*P* values for one way ANOVA.
[2]*P* values for Kruskal Wallis test.
[3]According to the results of the pairwise comparison test, in addition to group statistics, there is no statistically significant difference between groups with the same superscript letter (a/a or b/b), but there is a statistically significant difference between groups with different superscript letters (a/b).
[4]This column includes asthma patients who are neither atopic nor vaccinated.
ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; aPTT: Activated partial thromboplastin time; COVID-19: Coronavirus disease 2019; CRP: C-reactive protein; LDH: Lactate dehydrogenase.

When questioning the regular use of preventive/prophylactic asthma medication during COVID-19; 25% of our patients felt the need to use asthma medications. While 31% of patients needed salbutamol, none of our patients required prednol (methylprednisolone). The number of patients admitted to the emergency department was 20.6%. 5.7% of our patients were hospitalized (Figure 2B).

Comparison of the findings of vaccinated and unvaccinated patients

Our total vaccinated patients were 57/290 (19.6%). Among the vaccinated patients, fever symptoms were found at 47.4%, fatigue at 47.4%, and the number of patients with shortness of breath was 29.8%. Among the unvaccinated patients, 59% of patients described fever symptoms. While 36% of our patients complained of fatigue, 16% complained of shortness of breath (Table 1).

The rate of using salbutamol among vaccinated patients was 19.6%. The number of patients admitted to the emergency department is 14%. We had a total of 5.3% vaccinated patients hospitalized, and the need to use prednol was necessary in only 1/57 of our patients (Table 2). The average recovery time of the vaccinated patients was calculated as 9.1 d.

The rate of using salbutamol among unvaccinated patients was 26%. The number of patients admitted to the emergency department was 19%. A total of 5% of patients were hospitalized among unvaccinated patients, and the need for prednol was necessary for 3/228 of our patients (Table 2). In addition, the average recovery time of unvaccinated patients was calculated as 6.2 d.

DISCUSSION

In a review that included eight retrospective studies and 2914 children with COVID-19, asthma is one of the most common causes of comorbidity[5]. In the report published by the American CDC in 2020, the prevalence of asthma

Table 4 Evaluation of pathologic laboratory parameters in different groups of our asthma patients

	Others[1], (*n* = 169)	Vaccinated patients (*n* = 57)	Atopic patients (*n* = 64)	*P* value	ES
Leukopenia (< 4000)	8/67 (11.9)	2/12 (16.7)	4/17 (23.5)	0.541	0.125
Leukocytosis (> 10000)	11/67 (16.4)	0/12 (0)	1/17 (5.9)	0.193	0.186
Neutropenia (< 1500)	6/67 (9)	1/12 (8.3)	1/17 (5.9)	1.000	0.042
Neutrophilia (> 7500)	10/67 (14.9)	0/12 (0)	2/17 (11.8)	0.447	0.147
Lymphopenia (< 1500)	29/68 (42.6)	5/12 (41.7)	10/17 (58.8)	0.470	0.125
Lymphocytosis (> 4000)	3/68 (4.4)	0/12 (0)	2/17 (11.8)	0.475	0.152
Eosinophilia (> 500)	4/67 (6)	1/12 (8.3)	1/17 (5.9)	1.000	0.033
Thrombocytopenia (< 150000)	5/68 (7.4)	0/12 (0)	1/17 (5.9)	0.832	0.099
Thrombocytosis (> 450000)	1/68 (1.5)	0/12 (0)	0/17 (0)	1.000	0.067
Elevated CRP (> 3)	42/62 (67.7)	7/8 (87.5)	6/14 (42.9)	0.090	0.245
Elevated sedimentation (> 20)	1/5 (20)	0/2 (0)	-	1.000	0.258
Elevated AST (> 50)	2/63 (3.2)	0/12 (0)	1/16 (6.3)	1.000	0.097
Elevated ALT (> 50)	3/63 (4.8)	1/12 (8.3)	1/16 (6.3)	1.000	0.054
Elevated urea (> 45)	0/61	0/12	0/16	-	-
Elevated PT (>13.2)	3/33 (9.1)	0/4 (0)	2/8 (25)	0.261	0.221
Elevated aPTT (> 33.5)	2/31 (6.5)	0/4 (0)	0/7 (0)	1.000	0.133
Elevated D-dimer (> 500)	6/48 (12.5)	1/9 (11.1)	1/11 (9.1)	1.000	0.039
Elevated LDH (> 248)	15/47 (31.9)	2/8 (25)	4/12 (33.3)	1.000	0.052
Elevated total IgE (> 150)	10/37 (27)	1/2 (50)	9/13 (69.2)	**0.019**	0.376

[1]This column includes asthma patients who are neither atopic nor vaccinated.
Statistics were shown as *n*/total *n* (%). ES: Effect size (Cramer v coefficient).

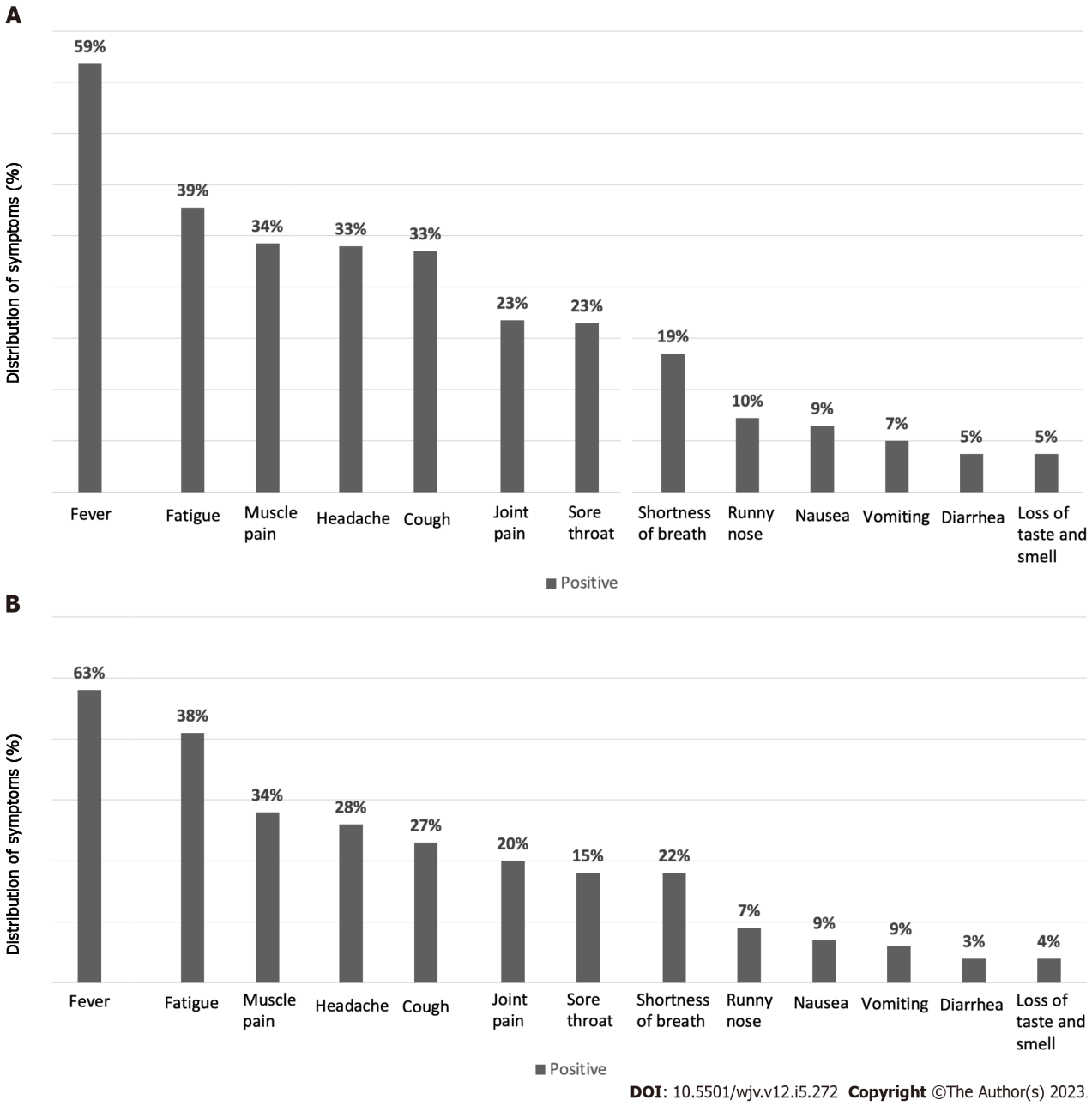
among those who had COVID-19 was reported as 5.8%[1]. In another study, which included 1802 patients with COVID-19, the rate of asthmatic patients was 7.8%[6]. It is known that the prevalence of asthma among COVID-19 patients is less common than in the general population[7,8].

After scanning the data of 5510 asthma patients over the age of 5, it was determined that 414 (7.5%) patients had COVID-19. In another study involving 43000 SARS-CoV-2 positive patients, asthma was reported as the most common comorbidity, with a prevalence of 10.2%[9]. In a study that involved 91 centers caring for approximately 133000 children with asthma, only 13/91 (14%) of the participating centers reported suspected cases of COVID-19 in children with asthma [10].

When we look at the vaccination rate of our patients, the vaccination rates among our patient group for individuals aged 12 and above and those aged 18 and above were determined to be 21.5% and 12.7% respectively. The vaccination rates in our study were lower than the current data because asthmatic patients in the childhood age group (17.18 ± 4.08 years) were included in the study, and the age limit for vaccination was lowered to 12 years in our country much later. In addition, as another factor, vaccine hesitancy at the time of the first use of vaccines is a known phenomenon that is still effective today. In a study that included 637 parents with children between 12 and 15 in the United States, vaccine hesitancy against COVID-19 vaccines was found in almost 1/3 of the parents[11]. A study conducted in our country stated that parents who were hesitant towards childhood vaccines also had a negative attitude toward COVID-19 vaccines[12]. In addition, in a study conducted with the parents of children with asthma in 2020, 19% of the participants (*n* = 309) stated that they did not consider vaccinating their children against COVID-19[13].

In our study, fever complaints were found at a rate of 59%, while this rate was lower in the literature compared to ours. In a study including 54 pediatric COVID-19 patients with asthma, complaints of fever were reported at a rate of 27.5%[5]. In a study comparing asthmatic COVID-19 patients and non-asthmatic COVID-19 patients, the rate of fever symptoms was 37.3% in the asthmatic group. Still, no significant difference was found between the two groups ($P = 0.55$)[7]. These data suggest that the rate of fever complaints in our study is higher than in other studies documented in the literature. When comparing vaccinated patients and unvaccinated patients regarding fever symptoms, no significant difference was observed between the two groups ($P = 0.012$).

The number of patients with cough complaints was found to be 33%. In a study including 54 COVID-19-positive asthma patients, the rate of cough (59.3%) was higher than in our study. In addition, the same study reported a significant difference in cough symptoms between the two groups with and without asthma ($P = 0.002$)[14]. In a study including 60 hospitalized asthma patients, it was reported that all asthma patients with COVID-19 (*n* = 10) had cough symptoms[15].



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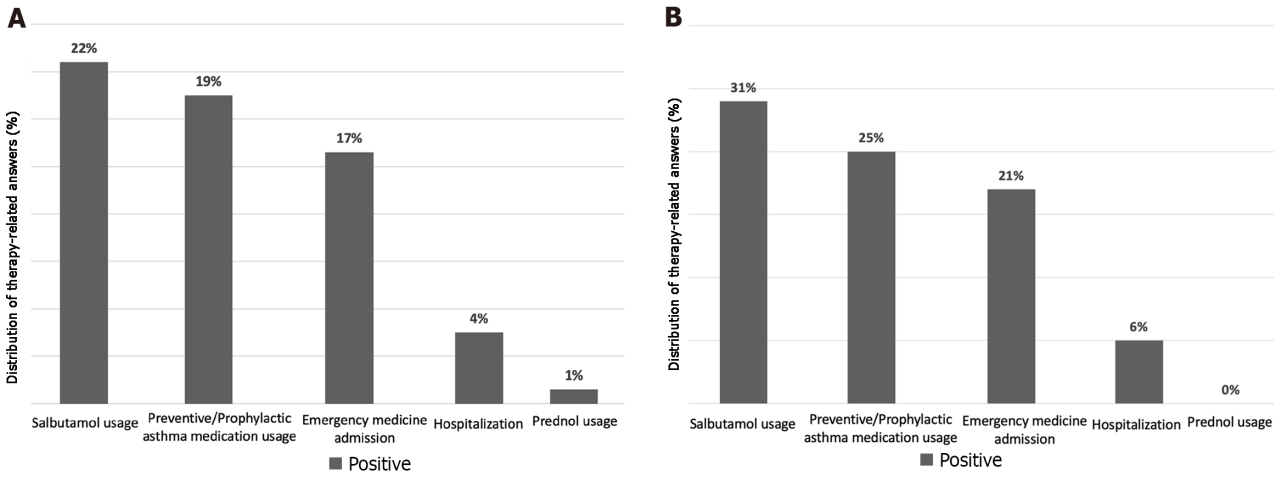
Figure 1 Symptom distributions of all patients. A: Distribution of symptoms according to the questions asked of patients and their answers; B: Distribution of symptoms according to the questions asked of patients with atopic asthma.

In our study, no significant difference was detected between the vaccinated and unvaccinated patient groups regarding cough complaints (P = 0.205).

When we look at the complaint of fatigue, the rate of 39% in our study was found to be higher compared to the literature. In one study, the rate of fatigue among COVID-19-positive patients with asthma was 16.9%[7]. Another study reported this rate as 16.7%[5].

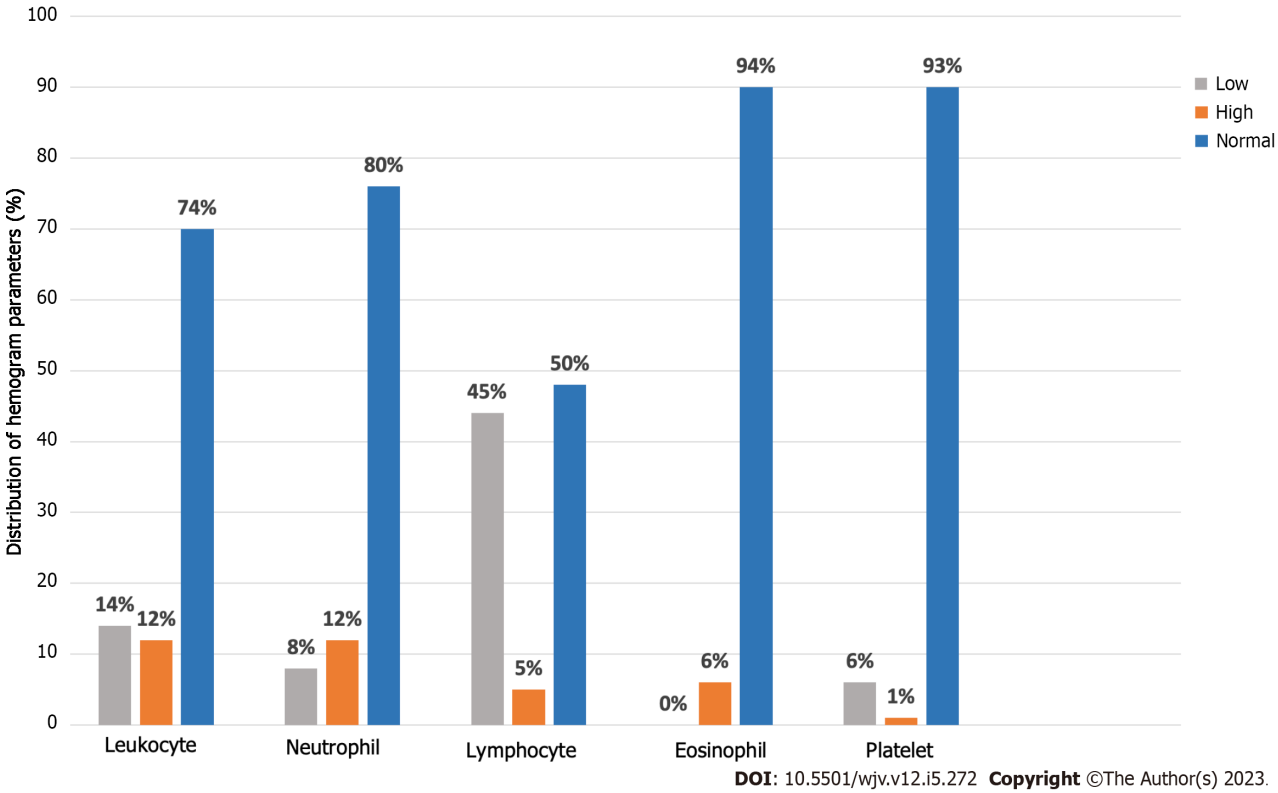
When we evaluated our vaccinated and unvaccinated patients in terms of symptom severity, it was found that 47% of vaccinated and 63% of unvaccinated patients had fever. It can be said that being vaccinated provides a 16% decrease in fever complaints, which seems significant (P = 0.008). When we look at fatigue complaints, vaccinated patients complained of fatigue 10% more often than non-vaccinated patients, a difference that can be considered significant (P = 0.002). In addition, dyspnea is 12% more common in vaccinated patients compared to unvaccinated patients (P = 0.041). When we look at the studies published in the literature, it has been reported that being vaccinated leads to a significant decrease in the frequency of symptoms, unlike the results we found. So, in a report published by the CDC, it was reported that vaccinated children had 60% fewer symptoms than those who were not vaccinated[16].

In our study, when the rate of asthma medication use was examined, we observed a rate of 21.2%. This rate was found to be lower compared to other studies in the literature. In a study conducted by Metbulut *et al*[5], the rate of asthma medication use was reported as 42.7%. When considering the rates of salbutamol usage, we found a rate of 22%, which was higher than that reported in other studies in the literature. In a study conducted by Gaietto *et al*[7], the rate of



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Figure 2 Asthma control parameters. A: Distribution of therapy-related answers to the questions asked to patients with asthma; B: Distribution of therapy-related answers to the questions asked to asthma patients with atopy (atopic/allergic asthma).

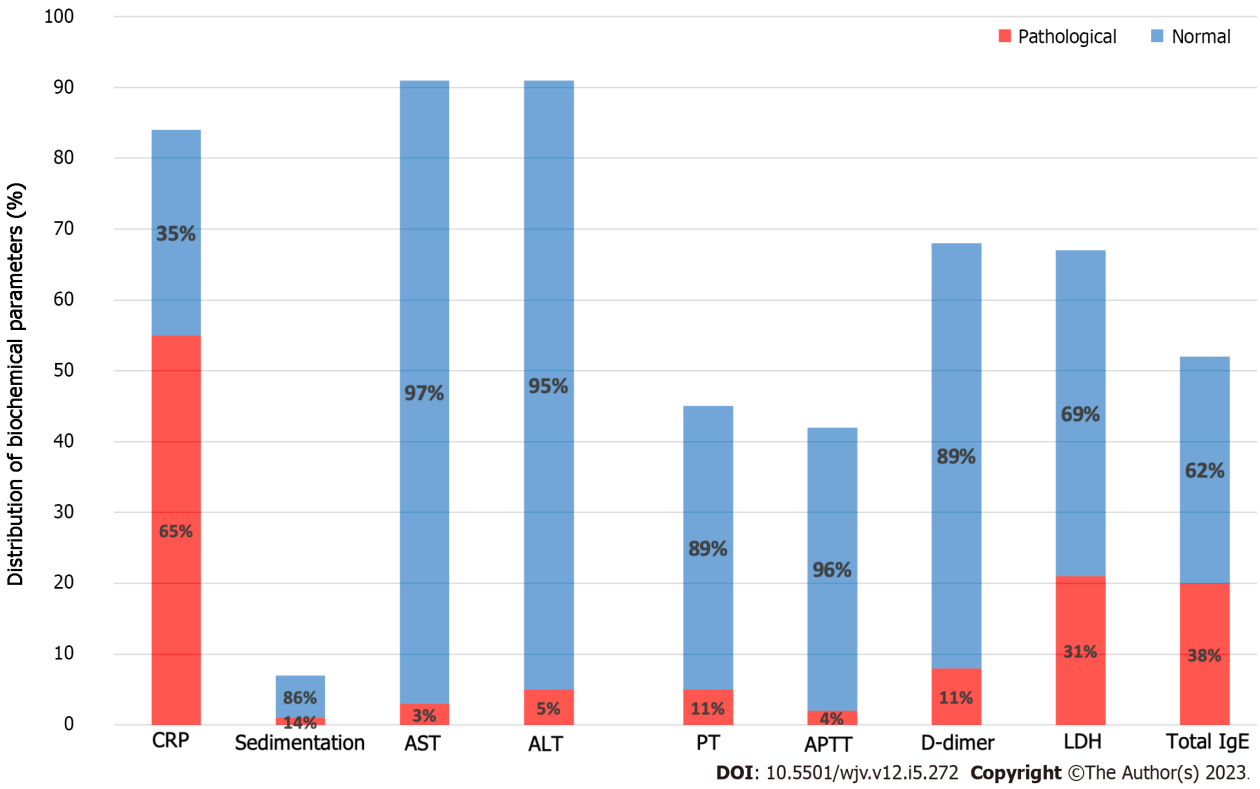


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Figure 3 Distribution of hemogram parameters.

Salbutamol usage among asthmatic COVID-19 patients was 17.6%. As expected, when regular maintenance medications are not used, asthma attacks tend to occur more frequently, leading to an increased usage of rescue medication, such as beta-agonists. No significant difference was observed in the asthma medication usage rate between vaccinated and unvaccinated patients (P = 0.957).

In our study, the rate of emergency department visits was determined to be 17.2%. Indeed, this rate appears to be similar to other studies in the literature. In one study, the rate of emergency department visits among asthmatic COVID-19 patients was 13.4%[7]. Another study demonstrated that implementing lockdown measures during COVID-19 significantly reduced the rate of emergency department visits in asthmatic patients[17]. The rate of methylprednisolone usage among our patients was determined to be 1%. As emergency department visits decrease, the usage rate of oral steroids, usually required during attacks, is also found to decrease. Vaccinated patients had 6% fewer emergency department visits than unvaccinated ones, but this difference was not statistically significant.



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Figure 4 Distribution of biochemical parameters.

Hospitalization rates were found to be 4% in our study. Indeed, this rate was similar to the rate (4.9%) in the study conducted by Gaietto *et al*[7]. Furthermore, in this study and other studies, asthma is a risk factor for hospitalization in SARS-CoV-2 infection[7,9]. In one study, the prevalence of asthma was reported as 34.2% in 34 hospitalized COVID-19 patients, and it was stated that COVID-19 did not show a serious course in asthma patients and required a lower level of care compared to other patients[18]. No significant difference was detected in terms of hospitalization between the vaccinated and unvaccinated patients (P = 0.692).

When examining the laboratory values of our patients, it was found that 14% exhibited leukopenia. While 10/14 (71%) of these patients had values in the normal range before COVID-19, a significant decrease was found in leukocyte counts during the period they had COVID-19. High leukocyte values were detected in one of our patients before contracting COVID-19. Based on these data, it appears that SARS-CoV-2 infection causes changes in patients' leukocyte counts. Leukopenia may be a common finding among patients with COVID-19, and a decrease in leukocyte count may inform the severity and course of the infection. However, it should be evaluated together with other clinical and laboratory data. In addition, in an article including 184 patients, leukopenia was found in 58 (34%) of the patients, but it is reported that only children with COVID-19 were screened in this article[14]. In a study comparing ten asthmatic patients with COVID-19 and 25 non-asthmatic patients, no significant difference was found in leukocyte counts[18]. No significant difference was observed in leukopenia frequency between our vaccinated patients and the unvaccinated patient group (P = 0.92).

When we look at our patients with leukocytosis (12%), the leukocyte count of only 23% of our patients was within the normal range before SARS-CoV-2 infection. There was no decrease in the rest of our patients, and an increase in leukocyte counts was detected before the infection. As a result, it was determined that the leukocyte count was not within the normal range and was already high in the majority of patients with leukocytosis in the period before SARS-CoV-2 infection. This situation requires careful evaluation of the effect of SARS-CoV-2 infection on leukocyte count, and the general clinical status and laboratory results of these patients should be evaluated together. In another article, it was reported that leukocytosis was detected in 194/610 (32%) pediatric moderately severe COVID-19 patients and 4/16 (25%) patients with severe COVID-19[19]. In another study comparing asthmatic patients with COVID-19 and patients without asthma, it was found that there was no significant difference between the two groups in terms of leukocyte values (P = 0.675)[20].

The number of patients with lymphopenia in our study was 44 out of 97 (45.3%), which is higher than other studies in the literature. In a study evaluating 66 COVID-19 patients aged between 6 and 17, lymphopenia was only observed in 2 out of 66 patients (3%)[21]. Another study, which included 486 hospitalized patients with confirmed SARS-CoV-2 infection, reported a prevalence of lymphopenia in 21% of the patients[22]. In a study comparing SARS-CoV-2 positive asthmatic patients (n = 54) with non-asthmatic patients (n = 162), no significant difference in serum lymphocyte levels was reported between the two groups (P = 0.263)[5].

When evaluating patients with neutropenia in our study, we observed neutropenia in only 8% of patients. Our findings appear to be consistent with the study conducted by Üzel *et al*[23]. In this study involving 59 patients, neutropenia was reported in 5 out of 59 patients (8.5%)[23]. However, it is worth noting that this study, unlike ours, specifically included

only children with COVID-19. Similar to our study, in a study comparing SARS-CoV-2 positive patients with and without asthma, no significant difference was found between the two groups in terms of serum neutrophil counts (P = 0.379)[5]. Another study comparing COVID-19 patients with and without asthma did not observe a significant difference between the two groups (P = 0.810)[20].

It can be stated that the platelet values of our patients yielded similar results compared to other studies in the literature. In our study, thrombocytopenia was observed in 6% of our 97 patients, while in a study conducted in Türkiye with 633 included patients, this rate was reported as 2%[24]. Furthermore, in the same article, the number of patients with thrombocytosis was 56 out of 633 (8.8%), whereas in our study, this rate was found to be 1% and represented a much smaller number of patients (n = 1)[24]. In another study comparing SARS-CoV-2 positive patients with and without asthma, no significant difference was found in platelet count (P = 0.480)[5].

When examining the CRP values of our patients, a significant elevation was observed in CRP levels in 54 out of 84 patients (65%). This rate is higher compared to other studies in the literature. In a study involving 633 patients, this rate was reported as 20%. The authors also noted that elevated CRP and other inflammatory markers may be associated with the severity of COVID-19[24]. In another study comparing SARS-CoV-2 positive patients with and without asthma, no significant difference was found in CRP values between the two groups (P = 0.523).

When evaluating LDH values, we found an elevation in LDH levels in 31% of our patients. This result is similar to the findings reported by Üzel *et al*[23]. Their study reported LDH elevation in 37.3% (n = 22) of the 59 symptomatic patients [23]. Another study compared LDH values between COVID-19 patients with asthma (n = 27) and without asthma (n = 42). This study found a significant difference in LDH values between the two groups (P = 0.035)[20].

When examining the D-dimer values of our patients, an elevation in D-dimer levels was observed in 8 out of 68 patients (11%). In a study involving 470 patients, D-dimer elevation was observed in 84 individuals (17.9%)[24]. Additionally, another article has linked elevated D-dimer levels and fibrinogen degradation products with COVID-19 mortality[25].

Comparison of symptoms in patients with and without atopy

When comparing the severity of COVID-19 in our patients with atopy and the other (nonatopic) group, we found that cough was observed in 27% of patients with atopy, while it increased to 32.5% in the other group; however, this difference was not statistically significant (P = 0.205). Fatigue was reported to be 1% more in patients with atopy compared to the other group. However, muscle pain was 10% more in other patients without atopy. Nevertheless, these two symptoms did not significantly differ (P = 0.365 and P = 0.296). Based on these findings, it is challenging to claim that systemic symptoms are a significant marker in patients with atopy. However, according to a study, the milder symptoms of COVID-19 in patients with atopy compared to those without atopy may be attributed to the hyperactivation of T cells in individuals with allergies[26]. Additionally, it is known that individuals with allergies who contract COVID-19 tend to have a milder course of the disease compared to those without allergies[27].

When considering the need for salbutamol, patients with atopy felt 11% more need than the other group; this difference was not statistically significant (P = 0.169). In our study, children with atopic asthma used asthma medications [inhaled corticosteroid (ICS)] 7% more than other group. However, this difference was not statistically significant (P = 0.372). In light of this information, it can be stated that patients with atopy experience an increase in asthma attacks when they contract SARS-CoV-2 infection. However, some studies in the literature have reported findings in favor of a decrease, rather than an increase, in asthma attacks during COVID-19, attributing the decrease in attack frequency to reduced exposure to allergens and outdoor inhaler risk factors for asthmatic children nationwide due to the precautions taken during the COVID-19 pandemic[26,28]. In our study, being atopic was suggested as a risk factor for triggering asthma attacks when children with atopy contracted SARS-CoV-2. When examining hospital admissions, those with atopy had 5% more emergency department visits compared to other group; however, this difference was not statistically significant (P = 0.485). Table 2 shows that the rates of emergency department visits and hospitalizations were higher but statistically insignificant in the atopic patients compared to the other group. Furthermore, it has been reported that COVID-19 does not progress to severe illness in patients with atopic asthma[29]. According to another study, overall hospital admission rates during the COVID-19 pandemic have dramatically decreased, but specific information about patients with atopic asthma was not shared in that study[29]. Another study reported that the rate of emergency department visits due to asthma decreased by 80% in 2020 compared to 2019 and 2018. Still, no patient atopic status data was provided[17].

When examining the biochemical and hematological parameters during the period of COVID-19 in patients with atopy and the other (nonatopic) group, it was found that patients with atopy had 10% less leukocytosis compared to the other group; however, this difference was not statistically significant (P = 0.193). In patients with atopy, a 12% higher rate of leukopenia was observed compared to the other group; however, we believe this difference is not significant (P = 0.541). In atopic children, elevated CRP values were found to be 25% less compared to the other group, and this difference was not statistically significant (P = 0.090). While lymphopenia, leukopenia, and elevated CRP values are known to be present in children with COVID-19, studies emphasize that these laboratory values are not specific to COVID-19[19].

Hospitalized vs non-hospitalized patients

When examining the symptoms of our patients who required hospitalization and experienced severe COVID-19, the most common complaints were fever and fatigue, with a prevalence of 57% and 40.6%; respectively. This was followed by headache and cough, with a prevalence of 35% and 33.6%; respectively. These data become more significant when comparing the group of hospitalized patients with those who were not. It was found that hospitalized patients had 23% more cough symptoms, but it was not statistically significant (P = 0.07). Hospitalized patients were seen to use 60% more salbutamol than non-hospitalized patients, and this difference was statistically significant. A positive correlation exists

between hospitalization and salbutamol use (P = 0.001). Hospitalized patients presented to the emergency department 34% more frequently; this difference was significant (P = 0.001). Additionally, a positive correlation exists between hospitalization and admission to the emergency department (P < 0.05). Hospitalized patients used methylprednisolone 13% more and asthma medications (ICS) 55% more compared to non-hospitalized patients, and these differences were statistically significant (P = 0.03 and 0.002, respectively).

When examining the acute phase reactants of our patients, elevated CRP levels were detected in 66%. These CRP values appear to be higher than in another study that included children with severe COVID-19 but are consistent[19]. Furthermore, leukopenia was observed in 17.3%, while leukocytosis in 7.3%. Neutropenia was found in 7.6%, and lymphopenia was found in 47.6%. Additionally, one patient had lymphocytosis and one patient had eosinophilia. Moreover, D-dimer elevation was observed in 11%, and LDH elevation was observed in 30%. D-dimer levels in the context of asthma and COVID-19 in children are crucial due to their association with coagulation activity and potential thrombotic complications. Increased D-dimer levels can function as indicators of heightened coagulation and fibrinolysis processes, potentially increasing the susceptibility of individuals to cerebrovascular events, including stroke[30]. Furthermore, research findings have indicated a notable correlation between D-dimer levels and the severity of the disease[31].

As many studies have indicated, asthma exacerbations did not increase in children during the COVID-19 period, and the implemented precautions and reduced allergen exposure have been reported to lead to a decrease in asthma symptoms[28,32]. According to our study, there is a correlation between the severity of COVID-19 and asthma symptoms and the course of the disease. However, it is worth noting that the retrospective nature of the study and the differences in sample size, age, and demographic characteristics between the two groups do not allow for an optimal comparison. Hospitalizations in children due to COVID-19 may increase asthma exacerbations, and asthma, which is shown as a risk factor in some data in the literature, is of importance[33]. Therefore, further investigation is needed to explore the relationship between COVID-19 and asthma, and it can be suggested that COVID-19 may trigger asthma attacks and asthma may impact the course of COVID-19.

Comparison of the findings of vaccinated and unvaccinated patients

When evaluating the symptom severity of the vaccinated and other (unvaccinated) group, it was found that 47% of vaccinated patients experienced fever. In comparison, the rate of fever complaints among the other group was 61%. It can be said that getting vaccinated resulted in a 14% decrease in fever complaints, which appears insignificant (P = 0.130). Regarding fatigue, vaccinated patients reported 11% more fatigue compared to the other group, but this difference is not significant (P = 0.365). Shortness of breath appears to be 15% more frequent in vaccinated patients compared to the other group. When examining the studies published in the literature, it is reported that vaccination leads to a significant decrease in symptom frequency, contrary to our findings. In fact, according to a report published by the CDC, vaccinated children experience the disease with 60% fewer symptoms compared to the unvaccinated[16].

As observed in Table 2, it can be seen that the rate of emergency department visits was lower in the vaccinated patients compared to atopic and other groups. Moreover, when we look at the control of asthma symptoms and hospitalization, no significant difference was observed between our study's groups of patients (vaccinated, atopic, and other [neither vaccinated nor atopic] group). Consistent with the findings of our study, a study conducted by Grandinetti *et al*[9] did not find any evidence supporting the hypothesis that COVID-19 vaccination exacerbates asthma attacks in asthmatic children. In our study, consistent with the previous findings, vaccinated patients experienced asthma exacerbations at a similar rate compared to the other group, and this difference did not appear to be statistically significant. The same study recommended deferring COVID-19 vaccination in patients with uncontrolled asthma until their clinical condition improves. A case study reported that an asthma patient who received the BNT162b2 (Pfizer-BioNTech) vaccine experienced an asthma exacerbation[34]. However, the authors noted that this study represents a single case, and further research is needed to conclude the general population.

Final a few points about the pathophysiology of COVID-19 and asthma

For a better understanding of the results of the study and the discussion, it would be appropriate to emphasize a few points about the pathophysiology of COVID-19 and allergic/non-allergic asthma diseases.

It is well known that asthma patients show reduced production of the antiviral interferon and lower ACE-2 expression. This is probably because ACE-2 expression is inversely correlated with type 2 (Th2: T helper 2) cytokine levels in atopic/allergic asthmatics. However, severe COVID-19 shows strong type I interferon expression early on. Consequently, this is inconsistent with the pathophysiology of COVID-19 disease development in asthma patients with a worse prognosis[8,27,29].

Although the impact of non-allergic and allergic asthma on the course of COVID-19 is often discussed in detail in the literature. It is reported that the prognosis of COVID-19 cases with common allergic diseases (atopic asthma, allergic rhinitis, atopic eczema, etc.) are not severe. Thus, the shift of the immune system to the Th2 phenotype in these patients may indicate a favorable balance in the pathogenesis of COVID-19 development[8,27,29].

CONCLUSION

According to our study, there is a correlation between the severity of COVID-19 and asthma symptoms and the course of the disease. However, it is worth noting that both the retrospective nature of the study and the differences in sample size, age, and demographic characteristics between the two groups do not allow for an optimal comparison. Therefore, further

investigation is needed to explore the relationship between COVID-19 and asthma, and it can be suggested that COVID-19 may trigger asthma attacks and asthma may impact the course of COVID-19.

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ARTICLE HIGHLIGHTS

Research background

Studies are ongoing as the consequences and impact of coronavirus disease 2019 (COVID-19) in children with chronic diseases such as asthma are controversial.

Research motivation

The consequences of COVID-19 in children with asthma are controversial.

Research objectives

This study aims to fill this research gap by retrospectively evaluating the course, laboratory, and clinical findings of COVID-19 among 414 asthmatic children followed up from the pediatric allergy outpatient clinic and known to have had COVID-19.

Research methods

The data of 5510 patients over the age of 5 diagnosed with asthma in our hospital's data were retrospectively scanned with certain parameters using protocol numbers from the hospital filing system.

Research results

As a result of retrospectively scanning the data of 5510 asthma patients over the age of 5, it was determined that 414 (7.5%) patients had COVID-19. The mean age of 414 patients was 17.18 ± 4.08 (min: 6; max: 28) years. 203 of our 414 patients are male, and 211 are female.

Research conclusions

According to our study, there is a correlation between the severity of COVID-19 and asthma symptoms and the course of the disease.

Research perspectives

Further investigation is needed to explore the relationship between COVID-19 and asthma, and it can be suggested that COVID-19 may trigger asthma attacks, and asthma may impact the course of COVID-19.

FOOTNOTES

Author contributions: Özata MC, Dikici Ü and Özdemir Ö designed the research; Özata MC and Dikici Ü performed the research; Dikici Ü and Özdemir Ö contributed analytic tools; Özata MC and Özdemir Ö analyzed the data; Özata MC, Dikici Ü and Özdemir Ö wrote the paper.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Use of inflammatory markers as predictor for mechanical ventilation in COVID-19 patients with stages IIIb-V chronic kidney disease?

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Abstract

BACKGROUND

Studies have shown elevated C-reactive protein (CRP) to predict mechanical ventilation (MV) in patients with coronavirus disease 2019 (COVID-19). Its utility is unknown in patients with chronic kidney disease (CKD), who have elevated baseline CRP levels due to chronic inflammation and reduced renal clearance.

AIM

To assess whether an association exists between elevated inflammatory markers and MV rate in patients with stages IIIb-V CKD and COVID-19.

METHODS

We conducted a retrospective cohort study on patients with COVID-19 and stages IIIb-V CKD. The primary outcome was the rate of invasive MV, the rate of noninvasive MV, and the rate of no MV. Statistical analyses used unpaired *t*-test for continuous variables and chi-square analysis for categorical variables. Cutoffs for variables were CRP: 100 mg/L, ferritin: 530 ng/mL, D-dimer: 0.5 mg/L, and lactate dehydrogenase (LDH): 590 U/L.

RESULTS

290 were screened, and 118 met the inclusion criteria. CRP, D-dimer, and ferritin were significantly different among the three groups. On univariate analysis for invasive MV (IMV), CRP had an odds ratio (OR)-5.44; ferritin, OR-2.8; LDH, OR-7.7; D-dimer, OR-3.9, (P < 0.05). The admission CRP level had an area under curve-receiver operator characteristic (AUROC): 0.747 for the IMV group (sensitivity-80.8%, specificity-50%) and 0.663 for the non-IMV (NIMV) group (area under the curve, sensitivity-69.2%, specificity-53%).

CONCLUSION

Our results demonstrate a positive correlation between CRP, ferritin, and D-dimer levels and MV and NIMV rates in CKD patients. The AUROC demonstrates a good sensitivity for CRP levels in detecting the need for MV in patients with stages IIIb-V CKD. This may be because of the greater magnitude of increased inflammation due to COVID-19 itself compared with increased inflammation and reduced clearance due to CKD alone.

Key Words: Coronavirus disease 2019; Chronic kidney disease; Inflammatory markers; C-reactive protein; Invasive mechanical ventilation; Non-invasive mechanical ventilation

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Core Tip: Our study demonstrates a positive correlation between the levels of inflammatory markers, including C-reactive protein, ferritin, and D-dimer, and the rate of invasive and non-invasive mechanical ventilation (MV) among coronavirus disease 2019 patients with chronic kidney disease (CKD), suggesting that these biomarkers are clinically useful to predict the need for MV in the CKD population.

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INTRODUCTION

A new variant of coronavirus lead to the pandemic of 2019 and was described as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Initially assumed to be a pathogen affecting the respiratory system, its effects have now been shown to be widespread affecting multiorgan infection and disease manifestation. With more than six million admissions and more than 1 million deaths, coronavirus disease 2019 (COVID-19) continues to be an infection with ongoing global concern. As we continue to discover the multitude of pathologies caused by the virus in different organ systems, the various associations and interactions between COVID-19 and existing chronic diseases slowly come to light. It was a sudden increase in the utility of inflammatory biomarkers, as they served as useful indicators of the severity of the underlying disease process. Severe COVID-19 disease is characterized by a hyperinflammatory condition, with multiorgan involvement due to a cytokine storm[1]. Multiple organ specific and nonspecific markers have been studied. Cardiac troponins, brain natriuretic peptide and multiple other markers have been shown to predict outcome in patients with and without cardiovascular disease[2,3]. Similar inflammatory markers including cytokines, including interleukin-6 (IL-6) and C-reactive protein (CRP), have been validated in multiple studies to help predict the severity of disease and the need for mechanical ventilation (MV)[4-6]. Studies have shown baseline elevation in these same inflammatory markers in patients with chronic kidney disease (CKD) alone, due to a chronic inflammatory milieu in chronic kidney disease and reduced renal clearance of these inflammatory markers[7]. Currently, the clinical utility of these inflammatory markers to predict the need for MV among patients with COVID-19 and underlying CKD is unclear. We aimed to assess if elevations in inflammatory markers can similarly predict the rate of invasive and non-invasive MV (IMV) (NIMV) among COVID-19 patients with CKD.

MATERIALS AND METHODS

Study design and participants

We conducted a retrospective single-center cross-sectional study of hospitalized patients between Dec 1, 2019, to Jan 1, 2022, at a 329-bed community teaching hospital in central Massachusetts. In order to be recruited into the study participants had to meet the inclusion criteria. Inclusion criteria: (1) Inpatients admitted with clinical symptomatology and subsequently diagnosed with SARS-CoV-2 infection with polymerase chain reaction test; (2) Age > 18 years; (3) Patients with history of stages IIIb-V CKD (estimated glomerular filtration < 45 cc/min as per National Kidney Foundation guidelines); and (4) Patients with documented inflammatory markers within 24 h of admission to the

hospital. Exclusion criteria included: (1) Pregnant patients; (2) Patients who had a history of renal transplantation; (3) Patients who required renal replacement therapy; and (4) Patients who failed to meet the inclusion criteria or if the required information could not be collected. The data was obtained by reviewing medical records, including demographic information, past medical history, medications, labs, and hospitalization course. Two independent physicians were involved with acquiring the data. All patient details were anonymized. Preformed proforma was used to acquire the study details such as age, sex, vaccination status, comorbidities such as hypertension, diabetes mellitus, chronic liver disease, chronic obstructive pulmonary disease, coronary artery disease, congestive heart failure and the use of medications such as steroids or remdesivir.

Exposure and outcomes

The primary endpoints measured included the rate of IMV, the rate of NIMV, and the rate of no requirement of mechanical ventilatory support (no-MV). As per American Thoracic Society guidelines, IMV was defined as intubation and provision of mechanical ventilatory support for respiratory failure. NIMV included bi-level positive airway pressure, high-flow oxygen, and continuous positive airway pressure support. No MV was defined as requiring oxygen *via* nasal cannula, oxymizer support, or those who did not require any oxygen supplementation. We assessed the levels of inflammatory markers among the three groups, including CRP, ferritin, lactate dehydrogenase (LDH), and D-dimer levels using certain cutoffs above which the levels were considered elevated. These cutoffs were designated as per institution protocol and was ≥ 100 mg/L for CRP, ≥ 530 ng/mL for ferritin, ≥ 590 U/L for LDH, and ≥ 0.5 mg/L for D-dimer respectively. We collected the baseline demographic data of the study population. Relevant clinical data associated with increased risk of MV, including a history of hypertension, diabetes mellitus, chronic liver disease, chronic pulmonary disease, coronary artery disease, and congestive heart failure, were collected. We also collected data regarding the different treatment modalities that each patient population received.

Ethical considerations: Institutional review board statement: The study was reviewed and approved by Saint Vincent-MetroWest Medical Center Institutional Review Board (approval No. 2020-035). Informed consent statement: The requirement of informed consent was waived by Saint Vincent- MetroWest Medical Center Institutional Review Board (approval No. 2020-035).

Data gathering and statistical analyses

The data was collected in Microsoft excel and was analyzed using SPSS. Non-parametric tests were employed since the data showed a non-normalcy distribution when we assessed it using the Shapiro-Wilk test. Chi-square analysis was employed for analyzing categorical variables and the Mann-Whitney U test was employed for analyzing continuous variables. Univariate logistic regression was utilized to assess the association between covariates and outcomes. We also calculated the area under the curve for invasive and NIMV for the different covariates, including CRP, ferritin, and LDH. The modalities of Medline, Pubmed and RCA were utilized to analyze high impact articles relevant to the current field of study and were incorporated in the discussion

RESULTS

Patient characteristics

Of the 290 patients screened, 118 met the inclusion criteria, among which 26 (22%) required IMV, 26 (22%) required NIMV, and 66 (56%) patients did not require any form of mechanical ventilatory support. There was an increased number of males in the group requiring IMV compared to those requiring NIMV (P = 0.01) (Table 1). Baseline demographics, including age > 60 years, vaccination status, and history of hypertension, diabetes mellitus, chronic liver disease, chronic pulmonary disease, coronary artery disease, and congestive heart failure, was similar among the three groups. In terms of medication administration, a significant difference was observed only in steroid use between patients on NIMV compared to those without (84.6% *vs* 66.7%, P = 0.01) (Table 1).

MV and inflammatory markers

The association between the levels of inflammatory markers and the use of invasive, non-invasive, and no mechanical ventilatory support was evaluated.

IMV: We observed a significant difference in the levels of inflammatory markers, including CRP (65.4% *vs* 25.8%, P = 0.01), ferritin (61.5% *vs* 36.4%, P = 0.01), troponin (42.3% *vs* 22.7%, P = 0.03), D-dimer (80.8% *vs* 51.5%, P = 0.01), and LDH (26.9% *vs* 4.5%, P = 0.04) between patients who required IMV and those who did not require MV (Table 2). This correlated with the significantly different mean levels of inflammatory markers observed between the two groups as well [CRP (160.2 *vs* 67, P = 0.001), ferritin (811 *vs* 295, P = 0.019), LDH (452 *vs* 321, P = 0.001) and D-dimer (2 *vs* 1, P = 0.001)]. Further univariate analysis between the inflammatory markers showed greater odds of having high inflammatory marker levels in patients who required IMV [CRP odds ratio (OR) 5.44, 95% confidence interval (CI): 2.04-14.48, ferritin (OR 2.8, 95%CI: 1.98-7.13), D-dimer (OR 3.95, 95%CI: 1.33-11.74), LDH (OR 7.73, 95%CI: 1.821-32.87), but troponin levels were not statistically significant (OR 2 .49, 95%CI: 0.947-6.56] (Table 3).

NIMV: A similar phenomenon of significantly different levels of inflammatory markers was observed in patients who required NIMV in comparison to those without mechanical ventilatory support requirements [CRP (53.8% *vs* 25.8%, P =

0.001), ferritin (65.4% *vs* 36.4%, *P* = 0.03), D-dimer (80.8% *vs* 51.5%, *P* = 0.01), and LDH (7.7% *vs* 4.5%, *P* = 0.001), but no significant difference was demonstrated in troponin levels (46.2% *vs* 22.7%, *P* = 0.06)] (Table 4). On assessing the mean levels of inflammatory markers between the two groups, we observed a significant difference in CRP (115.9 *vs* 67, *P* = 0.002), ferritin (628 *vs* 295, *P* = 0.013), and D-dimer (2 *vs* 1, *P* = 0.001) but no significant difference in LDH (357 *vs* 321, *P* = 0.29). We subjected these inflammatory biomarkers to univariate analysis, which showed increased odds of higher levels of all biomarkers except LDH among patients who required NIMV [CRP (OR 3.63, 95%CI: 1.30-8.67), ferritin (OR 3.306, 95%CI: 1.27-8.55), D-dimer (OR 3.95, 95%CI: 1.33-11.73), troponin (OR 2.94, 95%CI: 1.11-7.62) but no significant difference was demonstrated in LDH (OR 1.75, 95%CI: 0.27-11.12) (Table 5).

Area under curve-receiver operator characteristic (ROC) (AUROC): In order to further confirm the role of the inflammatory biomarkers in predicting the need for MV, ROC analysis was carried out. The AUROC for IMV was the following: for CRP, AUROC 0.747 (95%CI: 0.617-0.878, *P* = 0.001) that yielded a sensitivity of 80.8% and specificity of 50%; for ferritin, AUROC 0.658 (CI: 0.528-0.788, *P* = 0.019) with a sensitivity of 73% and specificity of 50%; for LDH, AUROC 0.699 (CI: 0.579-0.820, *P* = 0.003) with a sensitivity of 80.8% and specificity of 50%; and for D-dimer, AUC 0.751 (CI: 0.625-0.876, *P* = 0.001) with a sensitivity of 76.9% and specificity of 50% (Figure 1, Table 6).

The AUROC for NIMV was as follows: For CRP, AUROC 0.663 (95%CI: 0.527-0.799, *P* = 0.015) that yielded a sensitivity of 69.2% and specificity of 53%; for ferritin, AUROC 0.667 (CI: 0.555-0.778, *P* = 0.013) with a sensitivity of 80.8% and specificity of 53%; and for D-dimer, AUROC 0.740 (CI: 0.62-0.86, *P* = 0.004) with a sensitivity of 80.8% and specificity of 50% (Figure 2, Table 7).

DISCUSSION

This study is unique in assessing the utility of inflammatory markers, such as CRP, ferritin, LDH, and D-dimer in predicting the need for non-invasive as well as IMV in COVID-19 disease in patients with CKD. We observed that a higher proportion of COVID-19 patients with CKD who had elevated inflammatory marker levels ultimately required MV. The average inflammatory marker levels in all 3 groups (MV, NIMV and no MV) were high. Elevated levels of inflammatory markers were highly predictive of the need for IMV with corresponding AUROC of 0.747, 0.658, 0.699, and 0.751 for CRP, ferritin, LDH, and D-dimer, respectively. Although not all markers were predictive of the need for NIMV, CRP, ferritin, and D-dimer were predictive, with corresponding AUROCs of 0.663, 0.667, and 0.74, respectively. Although the pathophysiology explaining elevated LDH levels in patients requiring IMV but not amongst patients requiring NIMV is not explicitly clear, we hypothesize that this could be secondary to the LDH cutoff that was used to define levels as elevated. LDH enzyme plays a prominent role in active metabolism and levels are elevated with minor abnormalities such as tissue hypoxia and lysis necessitating a higher cutoff to detect significantly elevated LDH levels[8]. The results of our study reinforced the predictive value of CRP, ferritin, and D-dimer in patients with COVID-19 and underlying stages IIIb-V CKD. Among patients with CKD alone, studies have shown baseline elevated inflammatory marker levels, due to a chronic inflammatory milieu and decreased renal clearance of these inflammatory markers[7]. Our study highlighted the positive correlation of these markers with invasive as well as NIMV in COVID-19 patients with stages IIIb-V CKD; the high sensitivity of these markers demonstrated by the AUROC signifies their predictive potential.

In our study, the demographic variables were similar to the previous studies[4,5]. Male sex was associated with an increased risk of the need for invasive and NIMV. Sex may influence the severity of SARS-CoV-2 as the X-chromosome contains a higher density of immune-related genes and immunoregulatory elements related to innate and adaptive immunity[9]. There was an equal distribution of the need for MV in the presence of associated comorbidities, such as hypertension, diabetes mellitus, chronic liver disease, chronic obstructive pulmonary disease, coronary artery disease, and congestive heart failure. We noticed a significantly increased steroid administration rate in the NIMV group compared to the no MV group. One possible explanation for this finding could be the greater severity of the disease although there is no clear evidence to demonstrate the same

Biomarkers are a clinical reflection of the underlying disease process and help us assess the disease activity. This was frequently employed in COVID-19 disease with studies showing a correlation between elevated inflammatory marker levels and severe COVID-19 disease[5,6]. Although markers such as IL-6 were initially explored, they are cost-prohibitive and thus unsuitable for routine monitoring in COVID-19 patients[4]. This led to research on more routine biomarkers, including CRP, ferritin, LDH, and D-dimer, which have been shown to correlate well with the severity of COVID-19 disease[10]. Despite the use of different values of CRP to define elevation in multiple studies, such as Koozi *et al*[11] > 1000mg/L, Ryoo *et al*[12] > 140mg/L, and Liu *et al*[13] > 41.8 mg/L, there was a uniformly observed greater risk of severe COVID-19 disease[11-14].

Inflammatory markers are used for risk stratification and prognostication in several infectious diseases and malignancies, which are characterized by inflammation[15,16]. The pro-inflammatory nature of COVID-19 infection and associated organ dysfunction is well established[17,18]. Inflammatory markers, including CRP, erythrocyte sedimentation rate, LDH, and procalcitonin (PCT) are found to be elevated in patients with COVID-19[19,20]. Studies such as those by Herold *et al*[4] have demonstrated the utility of these biomarkers in prediction models that help detect the need for invasive and NIMV in patients with COVID-19 disease. They demonstrated an AUROC value of 0.97 and 0.86 for IL-6 and CRP with optimal cutoff values (IL-6: 80 pg/mL and CRP: 97 mg/L) that correctly classified 80% of their study population regarding their risk of respiratory failure[4]. The study by Li *et al*[5] used a multivariate stepwise logistic regression model to show the use of a glucocorticoid, increased neutrophil count, and PCT level in COVID-19 as predictive indicators for NIMV and the use of glucocorticoid increased neutrophil count and LDH level as effective predictors for

Table 1 Demographic information, *n* (%)

Variables	Invasive mechanical ventilation	Non-invasive mechanical ventilation	No mechanical ventilation	Total	*P* value[a]	*P* value[b]
Age > 60 yr	24	25	62	111	1.00	1.00
	92.3	96.2	93.9	94.1		
Male sex	18	12	26	56	0.01	0.55
	69.2	46.2	39.4	47.5		
Vaccinated against COVID-19	2	7	16	25	0.13	0.16
	7.7	26.9	24.2	21.2		
Hypertension	25	21	59	105	0.43	0.31
	96.2	80.8	89.4	89.0		
Diabetes mellitus	15	13	33	61	0.51	1.00
	57.7	50.0	50.0	51.7		
Chronic liver disease	0	1	1	2	1.00	0.49
	0.0	3.8	1.5	1.7		
Chronic obstructive pulmonary disease	8	11	13	32	0.26	0.26
	30.8	42.3	19.7	27.1		
Coronary artery disease	11	8	21	40	0.34	0.92
	42.3	30.8	31.8	33.9		
Congestive heart failure	10	11	17	38	0.23	0.12
	38.5	42.3	25.8	32.2		
Remdesivir	12	16	32	60	0.84	0.26
	46.2	61.5	48.5	50.8		
Steroids	24	22	44	90	0.01	0.12
	92.3	84.6	66.7	76.3		

[a]Chi square test between non mechanical ventilation and non invasive mechanical ventilation.
[b]Chi square test between non mechanical ventilation and invasive mechanical ventilation. COVID-19: Coronavirus disease 2019.

Table 2 Inflammatory marker levels between invasive mechanical ventilation and no mechanical ventilation, *n* (%)

Variables	Invasive mechanical ventilation	No mechanical ventilation	Total	*P* value[a]
CRP level (mg/L)	17	17	48	0.01
	65.4	25.8	40.7	
Ferritin level (ng/mL)	16	24	57	0.01
	61.5	36.4	48.3	
LDH level (U/L)	7	3	12	0.04
	26.9	4.5	10.2	
Troponin (ng/mL)	11	15	38	0.03
	42.3	22.7	32.2	
D-dimer (mg/L)	21	34	76	0.01
	80.8	51.5	64.4	

[a]Chi square test between non mechanical ventilation and non invasive mechanical ventilation. CRP: C reactive protein; LDH: Lactate dehydrogenase.

Table 3 Univariate analysis-invasive mechanical ventilation

Variables	OR	95%CI
CRP level	5.444	2.047-14.483
Ferritin level	2.8	1.098-7.138
LDH level	7.737	1.821-32.87
Troponin level	2.493	0.947-6.56
D-dimer level	3.953	1.331-11.74

CRP: C reactive protein; LDH: Lactate dehydrogenase; OR: Odds ratio; CI: Confidence interval.

Table 4 Inflammatory marker levels between non-invasive mechanical ventilation and no mechanical ventilation, *n* (%)

Variables	Non-invasive mechanical ventilation	No mechanical ventilation	Total	*P* value[a]
CRP level (mg/L)	14	17	48	0.001
	53.8	25.8	40.7	
Ferritin level (ng/mL)	17	24	57	0.03
	65.4	36.4	48.3	
LDH level (U/L)	2	3	12	0.001
	7.7	4.5	10.2	
Troponin (ng/mL)	12	15	38	0.06
	46.2	22.7	32.2	
D-dimer (mg/L)	21	34	76	0.01
	80.8	51.5	64.4	

[a]Chi square test between non mechanical ventilation and non invasive mechanical ventilation.
CRP: C reactive protein; LDH: Lactate dehydrogenase.

Table 5 Univariate analysis-non-invasive mechanical ventilation

Variables	OR	95%CI
CRP level	3.363	1.303-8.679
Ferritin level	3.306	1.277-8.55
LDH level	1.750	0.275-11.129
Troponin level	2.914	1.113-7.628
D-dimer level	3.953	1.331-11.736

CRP: C reactive protein; LDH: Lactate dehydrogenase; OR: Odds ratio; CI: Confidence interval.

IMV. In another single-center retrospective observational study, ferritin, LDH, absolute lymphocyte count, and CRP were found to predict the probability of early MIV with an accuracy of 88%[21].

The inflammatory markers are renally cleared, and hence reduced kidney function is associated with elevated levels of serum inflammatory markers. In addition, CKD is associated with chronic inflammation. Studies have demonstrated an elevation of CRP levels in patients with CKD and a negative correlation between CRP levels and glomerular filtration rate (GFR). There is evidence that inflammation, as measured by CRP level, increases with declining renal function in CKD patients[22-24]. A study by Keller *et al*[25] showed that in patients with initial stages of CKD and with end stage renal disease, the levels of CRP, fibrinogen, D-dimer, coagulation factor VII, factor VIII were increased, either due to increased production *vs* decreased clearance. CKD stages IIIb-V was selected since there was a significant increase in mortality rate amongst patients with CKD IIIb-V[26].

Table 6 Area under the curve-invasive mechanical ventilation

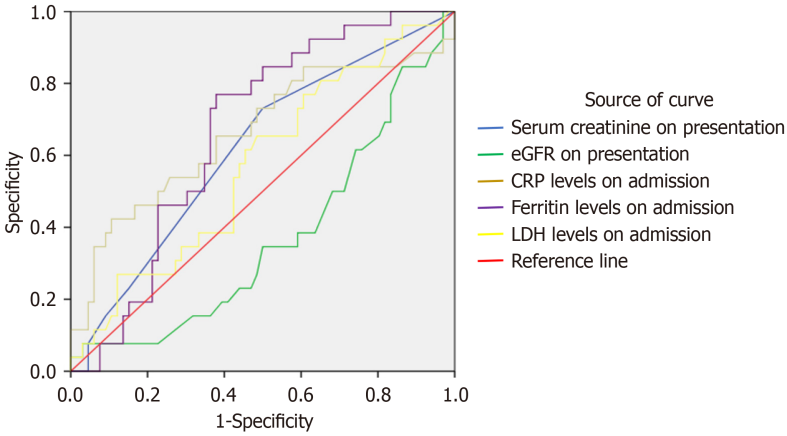
Variables on admission	AUC	*P* value	95% confidence interval		Sensitivity (%)	Specificity (%)
			Lower limit	Upper limit		
CRP level	0.747	0.001	0.617	0.878	80.8	51
Ferritin level	0.658	0.019	0.528	0.788	73	53
LDH level	0.699	0.003	0.579	0.820	80.8	51
D-dimer level	0.751	0.001	0.625	0.876	76.9	52

CRP: C reactive protein; LDH: Lactate dehydrogenase; AUC: Area under curve.

Table 7 Area under the curve-non invasive mechanical ventilation

Variables on admission	AUC	*P* value	95% confidence interval		Sensitivity (%)	Specificity (%)
			Lower limit	Upper limit		
CRP level	0.663	0.015	0.527	0.799	69.2	53
Ferritin level	0.667	0.013	0.555	0.778	80.8	53
LDH level	0.573	0.280	0.445	0.700	61.5	55
D-dimer level	0.740	0.0004	0.620	0.860	80.8	50

CRP: C reactive protein; LDH: Lactate dehydrogenase; AUC: Area under curve.

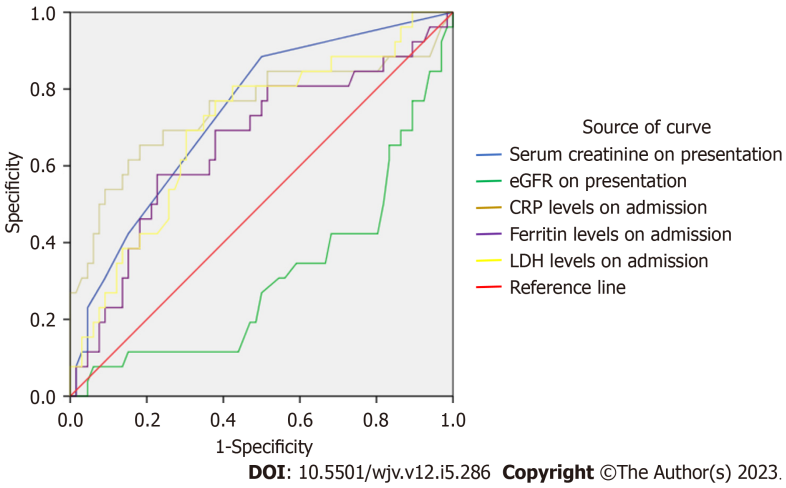


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Figure 1 Receiver operator characteristic curve for noninvasive mechanical ventilation. eGFR: Estimated glomerular filtration rate; CRP: C-reactive protein; LDH: Lactate dehydrogenase.

In our study, the mean CRP levels at admission in COVID-19 patients with stages IIIb-V CKD requiring IMV were remarkably higher than those who did not require MV (160.19 *vs* 67.02, $P = 0.001$). This finding likely reflects the impact of acute, severe COVID-19-related illness on the existing chronic inflammation in CKD, and concomitant reduced renal clearance of inflammatory markers. We found CRP, ferritin, LDH, and D-dimer to be good predictors of IMV and CRP, ferritin, and D-dimer to be good predictors of NIMV. Regardless of the negative correlation of inflammatory biomarkers with GFR in CKD, our study validated their high sensitivity in predicting COVID-19 prognosis in this specific population.

Limitations: One of the limitations of our study includes a small study population. We also did not include patients who had a history of renal transplantation, in order to minimize the influence of immunosuppressive medications in our study population. Another limiting factor includes the absence of information about baseline inflammatory marker levels in the setting of their underlying CKD. There are multiple factors that influence inflammatory marker levels, such as age, body mass index, sex, use of nicotine, blood pressure, and liver injury[20]. We did not study more specific markers such as IL-6, IL-1β, and IL-8, which are more sensitive but are cost-prohibitive in the real-world setting. We did not study the interactions of other comorbidities, interventions, and various medications with these inflammatory markers and the disease severity[27-29]. We also did not have the long term follow up details of these patients.



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Figure 2 Receiver operator characteristic curve for invasive mechanical ventilation. eGFR: Estimated glomerular filtration rate; CRP: C-reactive protein; LDH: Lactate dehydrogenase.

Future implications: Further prospective studies are needed to establish the correlation between the levels of inflammatory markers and the need for MV in COVID-19 patients with CKD. Validation of these inflammatory biomarkers is key in establishing their use as predictive indices. With the clinical utility of these inflammatory markers being described, it is imperative to study the impact of different disease processes on these inflammatory markers before employing them as clinical tools to guide the diagnosis and management of acute COVID-19 infection.

CONCLUSION

Our study explored the efficacy and predictive ability of inflammatory markers in detecting the risk of respiratory failure and the subsequent need for invasive and NIMV among COVID-19 patients with pre-existing CKD. We demonstrated that inflammatory markers, including CRP, ferritin, and D-dimer are useful predictive indicators of invasive and non-invasive MV in COVID-19 patients with stages IIIb-V CKD. The AUROC demonstrates good sensitivity for CRP levels in predicting the need for MV in the general population as well as in patients with stages IIIb-V CKD. This could be explained by the rationale that COVID-19 creates a greater magnitude of increased inflammation compared with increased inflammation due to CKD alone. With an increased need for better prognostic tools to help predict the severity of disease, especially among high-risk populations, and with the rising use of inflammatory markers to risk-stratify patients with COVID-19, large-scale, prospective studies are needed to delineate the optimal utilization of these biomarkers.

ARTICLE HIGHLIGHTS

Research background

Inflammatory markers have been validated in multiple studies to help predict the severity of disease and the need for mechanical ventilation (MV). Studies have shown baseline elevation in these same inflammatory markers in patients with chronic kidney disease (CKD) alone, due to a chronic inflammatory milieu in CKD and reduced renal clearance of these inflammatory markers. The clinical utility of these inflammatory markers to predict the need for MV among patients with coronavirus disease 2019 (COVID-19) and underlying CKD is unclear.

Research motivation

The use of biomarkers has been progressively increasing since the COVID-19 pandemic and the need for establishing the utility of these biomarkers in the presence of multiple comorbidities becomes essential to establish their clinical utility. Hence there is utmost need for this study to assess use of C-reactive protein level in assessing MV risk in CKD patients.

Research objectives

Since an increased level of inflammatory markers were observed in patients with chronic kidney disease, especially amongst those with stages IIIb-V, we planned to assess the utility of inflammatory biomarkers by evaluating the rate of MV and the levels of inflammatory biomarkers in stages IIIb-V chronic kidney disease patients who are diagnosed with COVID-19.

Research methods

In order to analyze the association between inflammatory marker levels and rate of MV, we did a single-center retrospective cohort study. The patients included in the study comprised of patients with stage IIIb-V CKD admitted to a community hospital with a diagnosis of COVID-19 infection. Amongst such patients, we extracted information regarding their inflammatory marker levels and their need for invasive and non-invasive MV (IMV) (NIMV) during their hospital stay.

Research results

A total of 290 patients were admitted between the study period of December 2019 to January, 2022 and amongst them 118 met the inclusion criteria. When we compared the rates of IMV, the group with IMV patients had a greater level of inflammatory markers. We also found a similar result when we compared the inflammatory marker levels amongst NIMV patients.

Research conclusions

Our results showed that elevated inflammatory marker levels were still associated with an increased rate of IMV and NIMV even amongst stage IIIb-V CKD patients with COVID-19 disease, thereby demonstrating the clinical utility of these biomarkers in assessing disease severity despite their baseline elevated levels observed in CKD patients.

Research perspectives

Validation of these inflammatory biomarkers is key in establishing their use as predictive indices. With the clinical utility of these inflammatory markers being described, it is imperative to study the impact of different disease processes on these inflammatory markers before employing them as clinical tools to guide the diagnosis and management of acute COVID-19 infection.

FOOTNOTES

Author contributions: Shanmugavel Geetha H and Martin S conceived the idea for the study; Shanmugavel Geetha H, GogtayM, Abraham GM, and Martin S designed and undertook the literature review; Shanmugavel Geetha H, Prabhu S, Sekar A, and Gogtay M collected data; Gogtay M and Singh Y performed the statistical analysis, figures, and appendix and analyzed and interpreted the data; Shanmugavel Geetha H, Prabhu S, Sekar A, Singh Y, and Gogtay M wrote the first draft of the manuscript; Shanmugavel Geetha H, Singh Y, Sekar A, Abraham GM, Martin S, Mishra AK and Gogtay M revised the subsequent drafts of the manuscript; all authors reviewed and agreed on the final draft of the manuscript.

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ORIGINAL ARTICLE

Basic Study

Perilipin2 inhibits the replication of hepatitis B virus deoxyribonucleic acid by regulating autophagy under high-fat conditions

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Abstract

BACKGROUND

Chronic hepatitis B virus (HBV) infection is often associated with increased lipid deposition in hepatocytes. However, when combined with non-alcoholic fatty liver disease or hyperlipidemia, it tends to have a lower HBV deoxyribonucleic acid (DNA) load. The relationship between lipid metabolism and HBV DNA replication and its underlying mechanisms are not well understood.

AIM

To investigate the relationship between lipid metabolism and HBV DNA replication and its underlying mechanisms.

METHODS

1603 HBsAg-seropositive patients were included in the study. We first explored the relationship between patients' lipid levels, hepatic steatosis, and HBV DNA load. Also, we constructed an HBV infection combined with a hepatic steatosis cell model *in vitro* by fatty acid stimulation of HepG2.2.15 cells to validate the effect of lipid metabolism on HBV DNA replication *in vitro*. By knocking down and overexpressing Plin2, we observed whether Plin2 regulates autophagy and HBV replication. By inhibiting both Plin2 and cellular autophagy under high lipid

stimulation, we examined whether the Plin2-autophagy pathway regulates HBV replication.

RESULTS

The results revealed that serum triglyceride levels, high-density lipoprotein levels, and hepatic steatosis ratio were significantly lower in the HBV-DNA high load group. Logistic regression analysis indicated that hepatic steatosis and serum triglyceride levels were negatively correlated with HBV-DNA load. Stratified analysis by HBeAg showed significant negative correlations between HBV-DNA load and hepatic steatosis ratio in both HBeAg-positive and HBeAg-negative groups. An *in vitro* cell model was developed by stimulating HepG2.2.15 cells with palmitic acid and oleic acid to study the relationship between HBV-DNA load and lipid metabolism. The results of the *in vitro* experiments suggested that fatty acid treatment increased lipid droplet deposition and decreased the expression of cell supernatant HBsAg, HBeAg, and HBV DNA load. Western blot and polymerase chain reaction analysis showed that fatty acid stimulation significantly induced Plin2 protein expression and inhibited the expression of hepatocyte autophagy proteins. Inhibition of Plin2 protein expression under fatty acid stimulation reversed the reduction in HBsAg and HBeAg expression and HBV DNA load induced by fatty acid stimulation and the inhibition of cellular autophagy. Knocking down Plin2 and blocking autophagy with 3-methyladenine (3-MA) inhibited HBV DNA replication.

CONCLUSION

In conclusion, lipid metabolism is a significant factor affecting HBV load in patients with HBV infection. The *in vitro* experiments established that fatty acid stimulation inhibits HBV replication *via* the Plin2-autophagy pathway.

Key Words: Lipid metabolism; Chronic HBV infection; Nonalcoholic fatty liver; Plin2; Autophagy

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Core Tip: Our data suggest that fatty acid stimulation inhibits hepatitis B virus (HBV) replication by upregulating Plin2 expression, inhibiting hepatocyte autophagy. This process associates with lipid metabolism, autophagy pathway, and HBV replication. Further study of lipid metabolism-Plin2-autophagy is important to understand HBV host interactions and pathogenesis better and suggests a possible route for treating patients with chronic HBV infection combined with nonalcoholic fatty liver disease.

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INTRODUCTION

Hepatitis B virus (HBV) infection is a global health issue[1]. According to the World Health Organization, about one-third of the world's population will contract acute HBV at some point in their life[2]. Current main treatments include nucleoside analogs and interferon; however, they are not effective in eliminating the virus[3]. Therefore, it is imperative to better understand the underlying mechanisms behind HBV infection-induced disease to find new targets for anti-HBV therapy.

The relationship between HBV infection and lipid metabolism has received more attention in the last decade. Clinical studies have demonstrated that chronic HBV infection enhances the incidence of nonalcoholic fatty liver disease (NAFLD), with NAFLD co-infected with HBV accounting for 13.5% of all HBV patients[4]. These studies indicate a close association between HBV infection and altered lipid metabolism. This present study intends to clarify the mechanism of why increased lipid deposition in the liver can also inhibited HBV replication in hepatocytes[5].

Autophagy is an evolutionarily conserved catabolic process that regulates HBV replication and is required to maintain cellular homeostasis in response to the microenvironment. It involves selective and non-selective mechanisms that cause intracellular substrate degradation[6]. HBV was found to be able to maintain its own replication by inducing hepatocyte autophagy, and when cellular autophagy was inhibited, HBV replication expression in hepatocytes was significantly reduced[7]. Perilipin2 (Plin2) is involved in the formation of lipid droplets in the liver and peripheral tissues[8]. Plin2 is highly upregulated in humans and rodents with NAFLD[5,9]. Purposeful knockdown of Plin2 protein in the mouse liver was found to significantly reduce liver weight, body weight, and adipose tissue mass[10]. In a previous study on NAFLD pathogenesis, it was found that Plin2 is not only involved in intracellular lipid deposition but also regulates intracellular autophagy, and stimulation of hepatocytes with high concentrations of fatty acids results in increased Plin2 expression and cellular autophagy inhibition[11].

Combined with the above, we speculated that the Plin2-autophagy pathway might be involved in regulating lipid deposition and HBV replication in hepatocytes. In this study, we first explored the relationship between patients' lipid levels, hepatic steatosis, and HBV deoxyribonucleic acid (DNA) load. Also, we constructed an HBV infection combined with a hepatic steatosis cell model *in vitro* by fatty acid stimulation of HepG2.2.15 cells to validate the effect of lipid metabolism on HBV DNA replication *in vitro*. By knocking down and overexpressing Plin2, we observed whether Plin2 regulates autophagy and HBV replication. By inhibiting both Plin2 and cellular autophagy under high lipid stimulation, we examined whether the Plin2-autophagy pathway regulates HBV replication. This present study intends to investigate the relationship between lipid metabolism and HBV replication through retrospective analysis and *in vitro* studies, providing a novel theoretical basis for the mechanism of HBV replication and a new target for searching new therapeutic sites.

MATERIALS AND METHODS

Ethical statement

The study has been approved by the Ethics Committee of Dalian Sixth People's Hospital. The privacy rights of human subjects were always respected during human experimentation, and informed consent was obtained prior to the experiment. The ethics program number is DLY/CB-IRB-026.

Patient selection

In this cross-sectional hospital-based study, all patients were recruited from the Dalian Sixth People's Hospital. A total of 1603 HBsAg-positive patients underwent a comprehensive health examination with no prior antiviral treatments to evaluate the effect of lipid profile on HBV viral replication. Additionally, 132 chronic hepatitis B patients were included in the study to investigate the effect of antiviral treatment on lipid profile. Patients with hepatitis C and D, autoimmune hepatitis, alcoholic fatty liver, Wilson's disease, drug-related hepatic steatosis, liver surgery, or liver transplantation were excluded from the study.

Clinical data collection

During the study, data on age, sex, alcohol consumption, and medical history were collected through face-to-face interviews. Following an overnight fast, blood samples were obtained from all participants. HBsAg, antibodies against HBsAg, HBeAg, antibodies against HBeAg, and antibodies against hepatitis B core antigen were measured using an immunoassay analyzer. The levels of serum HBV-DNA copy were measured using the COBAS Amplicor HBV monitor test (Cap/ctm, Roche, Switzerland). Clinical chemistry systems were used to evaluate the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase, total bile acids, total bilirubin, albumin, and bile acid.

Reagents and antibodies

The HBV-producing HepG2.2.15 hepatoma cell line with an integrated HBV genomic dimer was obtained from the Chinese Academy of Sciences (CAS) in Beijing, China. Trypsin-EDTA (#27250–018), fetal bovine serum (#10100147), phosphate-buffered saline (PBS) (#226013), Opti MEM medium (#22600134), and Dulbecco's modified essential medium (DMEM) (#31600083) were purchased from GIBCO BRL (Grand Island, NY, United States). Oleic acid (OA, #15724), palmitic acid (PA, #27567713), and 3-methyladenine (3MA, #M9281) were acquired from Sigma-Aldrich (Missouri, United States). Perilipin2 (Plin2, #ac219686) was obtained from Abcam Technologies (Abcam, Cambridge, United Kingdom). Light chain 3 (LC3, #A11280), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, #AC002), anti-rabbit IgG (#AS014), and anti-mouse IgG (#LV-AS003) were obtained from Wuhan Abcotec Biotechnology Co Ltd (Abclonal, Wuhan, China).

Cell line, plasmid, and transfection

HepG2.2.15 cells were obtained from the CAS and cultured in T25 cell culture flasks with 10% heat-inactivated fetal bovine serum (GIBCO BRL) (#10100147), antibiotics, and high glucose DMEM (GIBCO) (#31600083). The flasks were pre-cultured at 37°C for 18-24 h in a 5% CO_2 incubator.

Plin2 knockdown and overexpression plasmids were synthesized by Suzhou Jima Bio. Plasmid transfection was performed following the manufacturer's instructions for Lipofectamine 2000 transfection reagent (Invitrogen).

Western blotting

Whole-cell protein extracts were obtained by passive cell lysis using protease and phosphorylated protease inhibitors following the manufacturer's instructions, and protein concentrations were determined using the BCA method. SDS-PAGE (10% gel) was used to separate samples containing approximately 30 μg of protein per well, and the proteins were transferred to PVDF membranes, which were incubated with primary antibody at 4°C overnight after being closed in low-fat milk powder for 2 h at room temperature in TBST. The membranes were washed and placed in BLOTTO containing secondary antibodies (HRP-labeled goat anti-rabbit antibody) for 1.5 h at room temperature. Following TBST clearing, the membranes were placed in the chromogen for 30 s and exposed immediately to the exposure cassette. The method was to estimate the ratio of the brightness value of each sample strip to the brightness value of the corresponding GAPDH (internal reference) strip to get the corrected strip brightness value.

Triglyceride content detection

The triglyceride content of HepG2.2.15 was measured using the Triglyceride Quantification Assay Kit (Abcam) for colorimetric detection, following the manufacturer's protocol.

Real-time quantitative polymerase chain reaction analysis of mRNA expression

Several methods have been described for the detection of HBV daughter DNA in culture supernatants[12,13]. Real-time reverse transcription (RT) polymerase chain reaction (PCR) assay using primers 5'TCTTGCCTTACTTTTGGAAG 3' (forward) 5'AGTTCTTCTTCTTCTAGGGGACC3' (reverse) were used to measure HBV pgRNA and Plin2 mRNA levels in cells.

Assays to detect HBsAg and HBeAg in the cell culture supernatant

HBsAg and HBeAg levels were evaluated using a commercial ELISA kit (Hunan Shengxiang Biotechnology Co., Ltd., Hunan, China) following the manufacturer's instructions. The absorbance of each well was sequentially measured at 450 nm wavelength with zero blank air conditioning for the final assay.

Oil red staining

After stimulating HepG2.2.15 cells with free fatty acids, the cells were rinsed three times with PBS and fixed in 10% formalin for 15 min at room temperature. Following fixation, the cells were stained with Oil Red O for 20 min at room temperature. Stained cells were observed by a fluorescent microscope (Leica DMI 4000 B) on the white light setting (magnification, ×100).

GFP-LC3 fluorescence analysis

After preincubation in a complete medium at 37°C in 21% O_2 and 5% CO_2 for 24 h, the cells were transfected with GFP-LC3 following the manufacturer's instructions to monitor autophagy flux. After 8 h of transfection, the cells were rinsed with PBS; a complete culture medium was added to the cells. Finally, the samples were observed under a fluorescence microscope (Nikon, Tokyo, Japan)

Statistical analyses

Continuous variables were reported as mean ± standard deviation or median (interquartile range). Dependent variables were expressed as numbers or percentages. The Wilcoxon matched-pairs signed-rank test, a nonparametric statistical test, was employed to compare non-normally distributed continuous data. This test was used to analyze various variables, including age, gender, FBG, ALT, AST, ALP, γ-GGT, LDH, bile acids, total cholesterol, triglycerides, HDL-C, LDL-C, apoA, and apoB. Chi-square test was used to compare the differences in hepatic steatosis prevalence and the HBeAg seropositive prevalence of patients with high or low HBV DNA load. Binary logistic regression analysis was conducted to identify potential factors influencing HBV DNA load, such as hepatic steatosis, triglycerides, apoA, apoB, cholesterol, HDL-C, and LDL-C. Statistical analysis was performed using IBM Corp's SPSS version 24.0 software. A two-sided P value < 0.05 was considered statistically significant. All experiments were conducted in triplicates, and western blot data were analyzed using t-tests.

RESULTS

Clinical characteristics of HBsAg-seropositive participants

In this study, 1603 HBsAg-seropositive patients were included, of which 674 (42.0%) were HBeAg-seropositive. Of the total patients, 1015 (63.3%) were male, and 815 (50.8%) had a high HBV viral load, defined as serum HBV DNA levels > 10^4 copies/mL. The median age was 52 years (range 43–60). Table 1 presents the characteristics of the HBsAg-seropositive patients. Patients in the high HBV DNA group had a higher levels of ALT, AST, ALP, γ-GGT, LDH, and HDL-C, and lower levels of triglyceride (TG), FBG, albumin, LDL-C, and apoB compared to those in the low HBV DNA group (P < 0.05). However, there were no significant differences between the two groups with regards to TC, total bilirubin, and apoA. The characteristics of the HBeAg-seropositive patients are shown in Table 2.

Clinical characteristics of HBeAg-seropositive and HBeAg-seronegative patients

Out of the 1603 HBsAg-seropositive patients, 661 were HBeAg-seropositive and 942 were HBeAg-seronegative. Among the HBeAg-seropositive group, 460 (69.6%) patients had a high viral load while in the HBeAg-seronegative group, 355 (37.7%) patients had a high viral load. Patients with high viral loads in the HBeAg-seropositive group had higher levels of ALT, AST, and HDL-C, and lower levels of albumin, FBG, LDL-C, apoB, and a lower ratio of steatosis (P < 0.05) (Table 1). In contrast, patients with high viral loads in the HBeAg-seronegative group had higher levels of ALT, AST, ALP, albumin, total bilirubin, and bile acid, and lower levels of TC, LDL-C, apoB, γ-GGT, TG, and a lower ratio of steatosis (P < 0.05) (Table 2).

Metabolic factors associated with HBV-DNA load in the HBsAg-seropositive participants

Table 3 presents the results of binary logistic regression analyses of metabolic factors associated with HBV-DNA load. The serum levels of TG (OR 0.83, 95%CI 0.70–0.98, P = 0.027), apoA (OR 0.47, 95%CI 0.26–0.83, P = 0.009), and LDL-C (OR

Table 1 Demographic, clinical, and laboratory characteristics of HBsAg-positive patients with high and low viral load using 10^4 copies per mL as the cutoff point, *n* (%)

Factors	ALL	High HBV DNA	Low HBV DNA	*P* value
	Case *n* = 1603	Case *n* = 815	Case *n* = 788	
Demographic				
Age (yr)	52 (43–60)	51 (40–60)	53 (45–61)	0.010
Male gender	1015 (63.28)	131 (69.6)	401 (68.2)	0.703
Laboratory tests				
FBG (mmol/L)	5.10 (4.61–5.87)	5.00 (4.5–5.7)	5.21 (4.77–6.00)	< 0.001
ALT (U/L)	47.95 (25.00–109.92)	68.3 (37.1–155.83)	31.7 (20.3–67.7)	< 0.001
AST (U/L)	41.19 (25.00–81.97)	52.95 (33.33–106.8)	29.86 (21.31–55.55)	< 0.001
ALP (U/L)	81.40 (63.63–109.00)	85.30 (66.00–116.60)	76.65 (61.9–104.00)	0.003
γ-GGT (U/L)	51.20 (22.89–113.36)	60.00 (29.13–135.00)	41.60 (18.97–100.12)	0.038
LDH (U/L)	194.8 (170.23–231.10)	196.00 (171.56–239.00)	194.00 (168.94–224.61)	0.006
Albumin (g/L)	40.70 (35.33–44.70)	39.60 (33.02–43.51)	41.96 (37.49–45.80)	0.045
Total bilirubin (μmol/L)	16.53 (11.63–25.24)	17.60 (12.20–27.70)	15.39 (11.37–23.20)	0.165
Bile acids (μg/mL)	11.00 (5.18–30.00)	14.30 (6.30–34.20)	8.70 (4.26–23.88)	0.002
Total cholesterol (mmol/L)	4.25 (3.55–5.01)	4.19 (3.50–4.92)	4.37 (3.61–5.10)	0.517
Triglycerides (mmol/L)	1.06 (0.73–1.53)	1.02 (0.73–1.44)	1.12 (0.74–1.71)	< 0.001
HDL-C (mmol/L)	1.15 (0.90–1.41)	1.19 (0.91–1.43)	1.12 (0.89–1.38)	0.029
LDL-C (mmol/L)	2.40 (1.87–2.98)	2.27 (1.76–2.89)	2.51 (1.95–3.10)	0.018
apoA (g/L)	1.19 (1.01–1.38)	1.14 (0.99–1.27)	1.18 (1.02–1.39)	0.849
apoB (g/L)	0.87 (0.70–1.06)	0.84 (0.69–1.01)	0.91 (0.73–1.10)	0.002
Steatosis	163 (10.2)	25 (3.1)	138 (17.5)	< 0.001
HBeAg sero-positive	661 (41.2)	460(56.4)	201 (25.5)	< 0.001

HBV: Chronic hepatitis B virus; FBG: Fasting blood glucose; ALT: Alanine Aminotransferase; AST: Aspartateaminotransferase; ALP: Alkaline Posphatase; LDH: lactic dehydrogenase; HDL: High density lipoprotein; LDL: Low- density lipoprotein; apo-A: Apolipoprotein-A; apo-B: Apolipoprotein-B.

0.59, 95%CI 0.45–0.77, $P < 0.001$) were negatively associated with HBV-DNA load, while hepatic steatosis (OR 0.15, 95%CI 0.10–0.23, $P < 0.001$) was also negatively associated with HBV-DNA load. On the other hand, TC level (OR 1.39, 95%CI 1.09–1.77, $P = 0.009$) was positively associated with HBV-DNA load.

Metabolic factors associated with HBV-DNA load in HBeAg-seropositive and HBeAg-seronegative participants

Table 3 provides the results of logistic regression analyses of metabolic factors associated with HBV-DNA load in HBeAg-seropositive and HBeAg-seronegative patients. In HBeAg-seropositive patients, serum LDL-C level (OR 0.38, 95%CI 0.24–0.60, $P < 0.001$) and hepatic steatosis (OR 0.11, 95%CI 0.05–0.22, $P < 0.001$) were negatively associated with HBV-DNA load, while TC level (OR 2.33, 95%CI 1.55–3.51, $P < 0.001$) was positively associated with HBV-DNA load. In contrast, in HBeAg-seronegative patients, serum TG level (OR 0.69, 95%CI 0.55–0.87, $P = 0.002$), LDL-C level (OR 0.46, 95%CI 0.31–0.67, $P < 0.001$), and hepatic steatosis (OR 0.19, 95%CI 0.11–0.35, $P < 0.001$) were negatively associated with HBV-DNA load, while TC level (OR 1.69, 95%CI 1.19–2.40, $P = 0.004$) was positively associated with HBV-DNA load.

In vitro high-fat conditions inhibit the replication of HBV DNA

To investigate the relationship between lipid metabolism and HBV DNA replication *in vitro*, HepG2.2.15 cells were stimulated with varying concentrations of PA and oleic OA to create a model of HBV infection combined with hepatic steatosis. Real-time PCR was used to detect changes in HBV DNA replication levels. The results demonstrated that the expression load of HBV DNA significantly decreased in a concentration-dependent manner with the increase of PA or OA concentration (Figure 1A and B). The optimal stimulation concentration of OA was 0.2 M, while the optimal stimulation concentration of PA was 100 μmol/L. The optimal fatty acid concentrations were prepared into free fatty acids (FFA) at an OA:PA ratio of 2:1. After 72 h of FFA treatment, HepG2.2.15 cells were stained with oil red O hematoxylin, revealing a significant increase in intracellular lipid droplets in the FFA group compared to the control group, and fusion phenomena were observed (Figure 1C). The intracellular TG content was higher in the high-fat

Table 2 Clinical characteristics of HBeAg-seropositive and HBeAg-seronegative patients with high and low viral load using 10^4 copies per mL as the cutoff point, *n* (%)

Factors	HBeAg sero-positive			HBeAg sero-negative		
	High HBV DNA	Low HBV DNA	*P* value	High HBV DNA	Low HBV DNA	*P* value
	Case *n* = 460	Case *n* = 201		Case *n* = 355	Case *n* = 587	
Demographic						
Age (yr)	45 (36–59)	50 (40–59)	0.106	54 (45–61)	55 (46–61)	0.564
Male gender	314 (68.2)	137 (68.1)	0.412	128 (56.6)	93 (68.4)	0.026
Laboratory tests						
FBG (mmol/L)	4.83 (4.40–5.35)	5.10 (4.60–5.90)	< 0.001	5.23 (4.73–6.20)	5.28 (4.80–6.06)	0.795
ALT (U/L)	83.21 (43.45–190.62)	51.60 (26.62–120.07)	< 0.001	53.00 (3200–127.00)	27.60 (19.38–53.60)	< 0.001
AST (U/L)	59.30 (38.01–130.1)	46.85 (26.00–93.18)	< 0.001	46.50 (37.57–116.55)	27.00 (20.72–46.47)	< 0.001
ALP (U/L)	87.50 (67.98–117.09)	90.18 (67.00–127.15)	0.489	83.40 (65.20–111.90)	74.00 (60.00–96.00)	< 0.001
γ-GGT (U/L)	64.61 (33.50–139.92)	68.25 (33.25–145.87)	0.615	52.00 (21.90–115.69)	69.6 (33.40–164.30)	< 0.001
LDH (U/L)	195.00 (171.92–238.32)	193.40 (167.37–228.97)	0.38	197.00 (170.08–239.14)	194.00 (169.00–222.39)	0.077
Albumin (g/L)	39.40 (32.81–43.36)	40.30 (34.89–44.50)	0.026	54.00 (45.00–61.50)	43.90 (38.00–47.70)	< 0.001
Total bilirubin (μmol/L)	17.90 (12.40–28.35)	17.80 (13.21–32.52)	0.751	17.20 (11.9–27.00)	14.71 (11.08–22.47)	< 0.001
Bile acids (ug/mL)	16.60 (8.01—38.77)	16.50 (8.01–41.52)	0.904	10.06 (4.98–27.50)	7.07 (3.73–16.54)	< 0.001
Total cholesterol (mmol/L)	4.16 (3.50–4.85)	4.00 (3.30–4.83)	0.674	4.22 (3.49–5.01)	4.46 (3.71–5.15)	0.019
Triglycerides (mmol/L)	1.03 (0.73–1.44)	1.08 (0.71–1.71)	0.429	1.04 (0.73–1.41)	1.14 (0.75–1.72)	0.002
HDL-C (mmol/L)	1.19 (0.90–1.43)	1.08 (0.81–1.40)	0.041	1.20 (0.92–1.44)	1.12 (0.91–1.38)	0.13
LDL-C (mmol/L)	2.25 (1.76–2.90)	2.41 (1.89–3.06)	0.041	2.31 (1.72–2.87)	2.54 (1.97–3.12)	< 0.001
apoA (g/L)	1.17 (0.97–1.35)	1.15 (0.92–1.30)	0.204	1.21 (1.00–1.40)	1.20 (1.04–1.41)	0.624
apoB (g/L)	0.85 (0.69–1.02)	0.91 (0.70–1.12)	0.01	0.83 (0.68–1.00)	0.91 (0.74–1.09)	< 0.001
Steatosis	11 (2.4)	37 (18.4)	< 0.001	11 (3.0%)	101 (17.2)	< 0.001

HBV: Chronic hepatitis B virus; FBG: Fasting blood glucose; ALT: Alanine Aminotransferase; AST: Aspartateaminotransferase; ALP: Alkaline Posphatase; LDH: Lactic dehydrogenase; HDL: High density lipoprotein; LDL: Low- density lipoprotein; apo-A: Apolipoprotein-A; apo-B: Apolipoprotein-B.

stimulation conditions than in the control group (Figure 1D). ELISA was used to measure HBsAg and HBeAg levels in cell culture supernatants, and it was found that high-fat stimulation inhibited the expression of both HBsAg (Figure 1E) and HBeAg (Figure 1F).

Based on these findings, it can be concluded that high-fat conditions inhibit the expression of HBV DNA and its serum markers in a concentration-dependent manner. However, the exact mechanism underlying this inhibition remains unclear.

Fatty acids stimulation promoted the expression of Plin2 and inhibited the replication of HBV DNA

To investigate the role of Plin2 in inhibiting HBV replication under high lipid conditions, we performed both Plin2 knockdown and overexpression experiments in HepG2.2.15 cells after fatty acid stimulation. After downregulating Plin2 protein, the number and volume of intracellular lipid droplets significantly decreased in both the control group and FFA-stimulated group under microscopy, whereas the number of lipid droplets increased in the Plin2 overexpression group (Figure 2A). Additionally, TG content was observed to increase in the Plin2 overexpression group (Figure 2B and C). HBV DNA load increased significantly after knockdown of Plin2 (Figure 2D-F). The expression of HBsAg and HBeAg was also significantly upregulated after transfection with siPlin2 plasmid, whereas the expression of both markers was downregulated after overexpression of Plin2 (Figure 2G-J).

These findings suggest that high lipid conditions upregulate Plin2 expression and that Plin2 plays a role in counteracting the regulation of lipid metabolism.

Plin2 affects HBV DNA replication by regulating autophagy

Western blotting was used to examine the expression of Plin2 and autophagy-related proteins LC3-II and LC3-I. The

Table 3 Results of univariate and multivariate analyses on lipid level and steatosis and chronic hepatitis B viru deoxyribonucleic acid load

	P value	OR (95%CI)
HBsAg sero-positive		
Steatosis	< 0.001	0.15 (0.10–0.23)
Triglyceride	0.027	0.83 (0.70–0.98)
apoA	0.009	0.47 (0.26–0.83)
apoB	0.905	0.99 (0.91–1.08)
Cholesterol	0.009	1.39 (1.09–1.77)
HDL-C	0.171	1.38 (0.87–2.18)
LDL-C	< 0.001	0.59 (0.45–0.77)
HBeAg sero-positive		
Steatosis	< 0.001	0.11 (0.05–0.22)
Triglyceride	0.077	0.74 (0.53–1.03)
apoA	0.846	1.10 (0.41–2.96)
apoB	0.5	0.69 (0.23–2.02)
Cholesterol	< 0.001	2.33 (1.55–3.51)
HDL-C	0.204	0.61 (0.29–1.30)
LDL-C	< 0.001	0.38 (0.24–0.60)
HBeAg sero-negative		
Steatosis	< 0.001	0.19 (0.11–0.35)
Triglyceride	0.002	0.69 (0.55–0.87)
apoA	0.209	0.62 (0.29–1.31)
apoB	0.713	1.02 (0.91–1.14)
Cholesterol	0.004	1.69 (1.19–2.40)
HDL-C	0.904	0.96 (0.51–1.81)
LDL-C	< 0.001	0.46 (0.31–0.67)

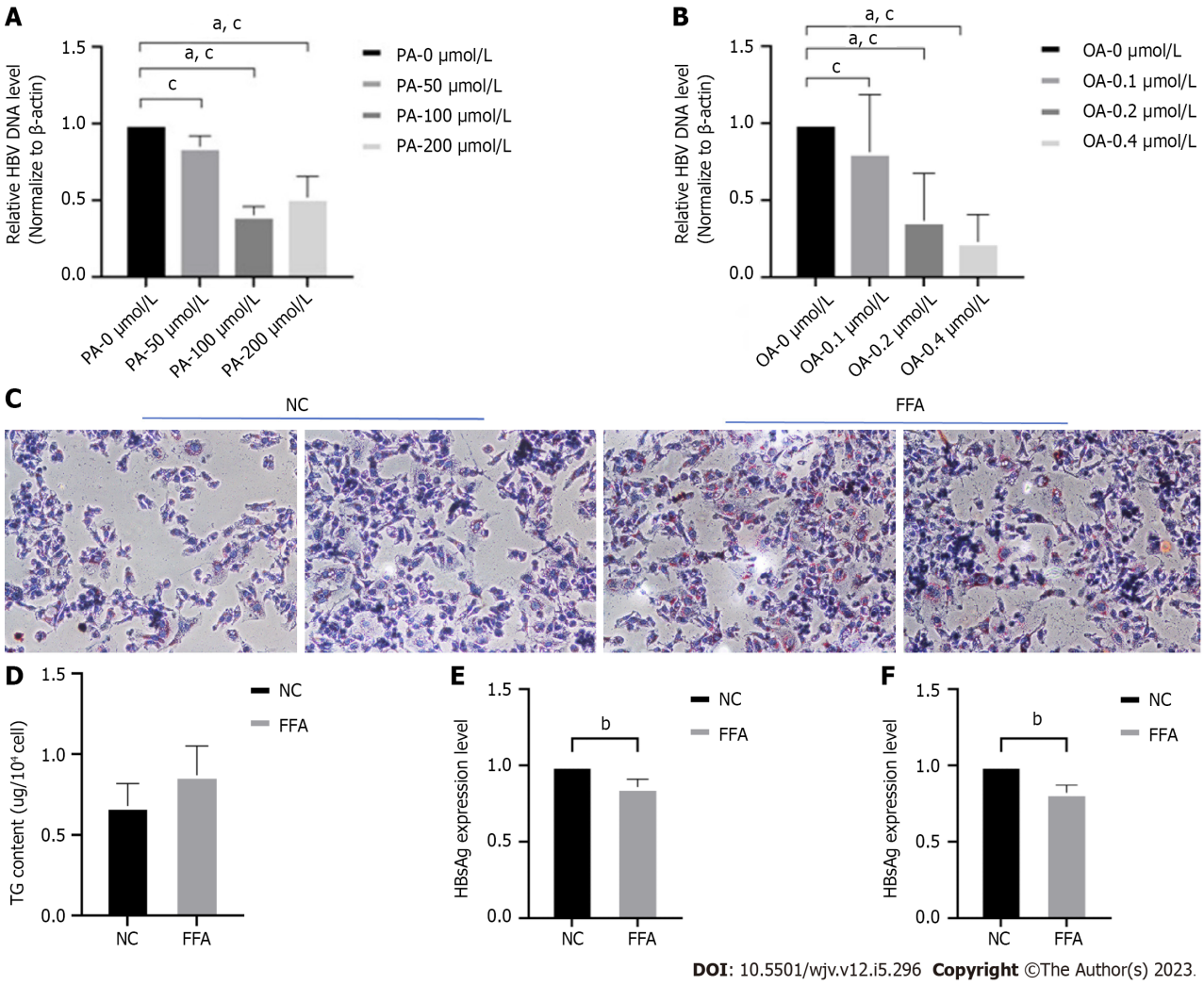
HBV: Chronic hepatitis B virus; FBG: Fasting blood glucose; ALT: Alanine Aminotransferase; AST: Aspartateaminotransferase; ALP: Alkaline Posphatase; LDH: Lactic dehydrogenase; HDL: High density lipoprotein; LDL: Low- density lipoprotein; apo-A: Apolipoprotein-A; apo-B: Apolipoprotein-B.

results showed that Plin2 expression was significantly upregulated under fatty acid stimulation conditions, and Plin2 knockdown under fatty acid stimulation conditions demonstrated a decreasing trend in upregulated Plin2 expression (Figure 3A-C). Autophagy-related proteins LC3-II/LC3-I were significantly decreased when HepG2.2.15 cells were stimulated with fatty acids, indicating autophagy inhibition. Autophagy expression increased when Plin2 expression was disturbed; after knockdown of Plin2 under high-fat conditions, autophagy was restored, whereas overexpression of Plin2 under both normal medium and high-fat stimulation significantly inhibited autophagy (Figure 3B and D). The number of autophagic vesicles significantly decreased when HepG2.2.15 cells were stimulated with fatty acids, indicating that autophagy was inhibited as seen through GFP-LC3 staining. The number of autophagic vesicles increased when Plin2 expression was interfered with and significantly decreased when Plin2 expression was overexpressed (Figure 3E).

Based on these findings, it can be concluded that fatty acid stimulation alters the autophagic trend by affecting the expression of Plin2, thereby affecting HBV DNA replication.

Lipid metabolism affects HBV DNA replication through Plin2autophagy-related pathway

Plin2 knockdown under fatty acid stimulation was observed to restore the inhibited HBV replication, whereas the restored DNA expression load was again inhibited after adding 3-MA to inhibit autophagy (Figure 3F). Plin2 protein expression remained unchanged upon the addition of autophagy inhibitor, as observed through Western blot analysis (Figure 3G and H). In contrast, the values of autophagy-related protein LC3-II/LC3-I were significantly downregulated (Figure 3G and I), indicating that autophagy was clearly inhibited. These results suggest that fatty acid stimulation inhibits autophagy and HBV DNA replication *via* Plin2.



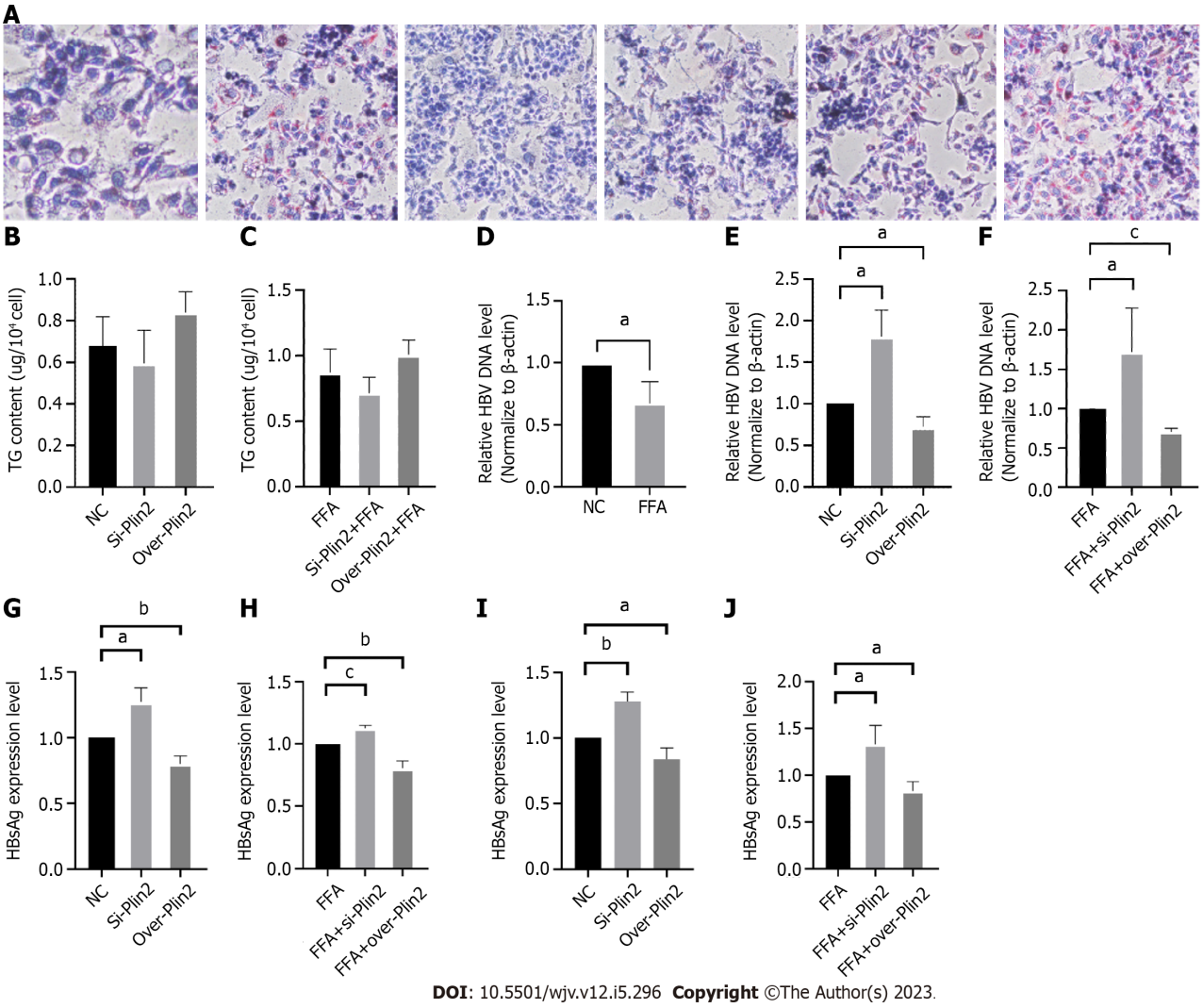
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Figure 1 *In vitro*, high lipid cases promote lipid droplet formation and inhibit chronic hepatitis B virus deoxyribonucleic acid replication and the choreographing of related antibodies. A and B: HepG2.2.15 cells were stimulated with different concentrations of palmitic acid (PA) and oleic acid (OA) for 48 h, and the expression of chronic hepatitis B virus deoxyribonucleic acid was detected; C: 0.2 mmol/L concentration of OA and 100 μmol/L concentration of PA were applied to stimulate HepG2.2.15 cells for 48 h, and the intracellular lipid droplet formation was detected by applying oil red O staining method; D: Detection of intracellular triglyceride content in both groups; E and F: After applying free fatty acids stimulation for 48 h, the levels of HBsAg and HBeAg secreted by the two groups of cells were detected by the ELISA method, respectively. [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$. NC: Nucleocapsid protein; FFA: Free fatty acids.

DISCUSSION

HBV, a DNA virus that causes immune-mediated liver disease, can be transmitted through blood and body fluids. Although immunomodulatory drugs such as interferon and antiviral drugs have a good safety profile and are also effective in controlling HBV replication in patients with chronic hepatitis B, they rarely eliminate HBV completely and do not completely eliminate liver cancer risk[14]. Therefore, there is an urgent requirement to explore the deeper regulatory mechanisms of HBV in order to seek the development of new drugs for HBV clearance.

Hepatic steatosis is characterized by an excessive accumulation of triglycerides in hepatocytes[15]. In previous studies, hepatic steatosis has been found in patients with chronic HBV infection, causing a reduction in their response to antiviral therapy[16]. However, there is mixed evidence on the association between hepatic steatosis and HBV replication. Several studies have shown that hepatic steatosis induces HBsAg clearance and reduces HBV replication[17,18]. Chia-Ming Chu's study showed that in patients with increased body mass index, hepatic steatosis accelerated HBsAg serological clearance by approximately 5 years[19]. Conversely, Lesmana *et al*[20] found no difference in HBV replication between HBV patients with and without hepatic steatosis. Our results indicated that serum triglycerides, HDL levels, and the rate of hepatic steatosis were significantly lower in the HBV-DNA high load (815 cases) group compared to the HBV-DNA low load (788 cases) group. The findings of logistic regression demonstrated that serum TG levels (OR 0.83 95%CI 0.70–0.98, $P = 0.027$), apoA levels (OR 0.47, 95%CI 0.26–0.83, $P = 0.009$), LDL-C levels (OR 0.59, 95%CI 0.45–0.77, $P < 0.001$) and hepatic steatosis (OR 0.15, 95%CI 0.10–0.23, $P < 0.001$) showed significant negative correlation with HBV-DNA load. As a result, we conducted a stratified analysis according to HBeAg serostatus. The results of logistic regression showed that hepatic steatosis serum triglyceride load was negatively correlated with blood HBV-DNA load in both HBeAg positive or negative groups ($P < 0.001$). These findings are consistent with a study reported by Jarcuska *et al*[21], which stated a

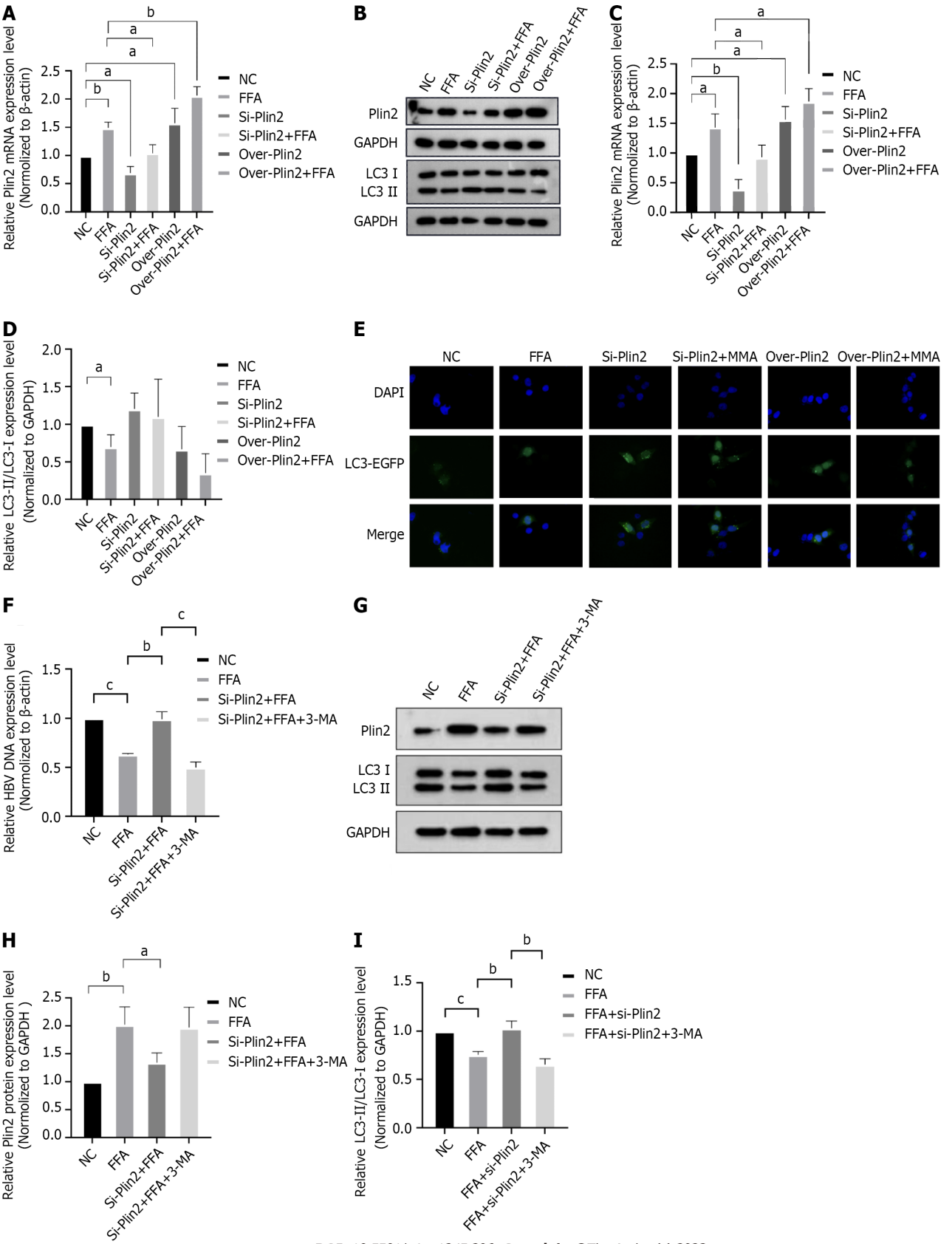


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Figure 2 To investigate the role of Plin2 in the effect of high-lipid conditions on chronic hepatitis B virus. A: Oil red O staining to observe lipid droplet formation; B and C: Triglyceride (TG) assay kit was applied to detect the TG content of each group; D-F: Quantitative polymerase chain reaction method was applied to detect the chronic hepatitis B virus deoxyribonucleic acid content in each group; G-J: HBsAg and HBeAg levels were detected in each group by the ELISA method. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.

significantly lower HBV-DNA load in patients with hypertriglyceridemia. These findings indicate that increased lipid metabolism in the body can inhibit HBV replication.

Previous studies have demonstrated that autophagy is closely associated with HBV DNA replication, and various factors in HBV infection, such as interferon Alpha and endoplasmic reticulum stress, can affect HBV replication by inducing autophagy[22]. Yongjun Tian *et al*[23] found that after liver-specific Atg5 knockdown in the HBV Tg05 mouse, the serum levels of HBeAg and HBsAg were decreased by about 50% and 60%, respectively. However, this autophagy inhibition decreased HBV DNA levels by more than 90%. A previous study showed that IFNα-2a treatment promoted autophagy initiation and blocked autophagy degradation, leading to a slight enhancement of HBV replication[24]. Autophagy disorders often result in metabolic abnormalities and play an essential role in the pathogenesis of numerous metabolic liver diseases, such as alcoholic liver disease and NAFLD[25]. Singh *et al*[26] coined the term "lipophagy" after identifying autophagy-mediated lipolytic functions in the LIPA pathway; TSAI T H showed that specific knockdown of Plin2 decreased triglyceride levels in mice by approximately 60%[27]. In another study of liver-specific Plin2 knockout mice, a significant increase in LC3 and p62-positive spots were detected in the livers of Plin2-deficient mice fed WTD[28]. In the study by Tsai *et al*[27], it was found that the down-regulation of Plin2 stimulated TG catabolism by upregulating autophagy expression through direct knockdown of Plin2. Therefore, we speculated that the high-fat environment regulates the Plin2-autophagy pathway and thus inhibits HBV DNA replication. Our experiments first examined the relationship between Plin2 and autophagy. Autophagy expression increased both under normal medium and under high-fat stimulation when Plin2 protein expression was down-regulated, and overexpression of Plin2 protein resulted in significant inhibition of autophagy. Fluorescence microscopy showed an increase in autophagy vesicles when Plin2 protein expression was down-regulated. Then the association between Plin2 and autophagy under fatty acid stimulation was further explored. Plin2 expression was significantly upregulated in HepG2.2.15 cells, and cellular autophagy was significantly inhibited. Compared to the control group, the expression of supernatant HBV DNA load and serological



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Figure 3 Abnormal lipid metabolism affects chronic hepatitis B virus deoxyribonucleic acid replication through the Plin2-autophagy-related pathway. A: Quantitative polymerase chain reaction (q-PCR) method was applied to detect Plin2 mRNA levels; B-D: Western blot to detect the expression of Plin2 and LC3 in each group and detect the grayscale value; E: GFP-LC3 formation was observed under the fluorescence microscope; F: Chronic hepatitis B virus deoxyribonucleic acid levels were detected by applying q-PCR; G-I: Western blot was performed to detect the expression of Plin2 and LC3 in each group and to detect the grayscale values. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.

markers in HepG2.2.15 cells were significantly lower in the Plin2 knockdown group, whereas the overexpression of Plin2 was reversed. Finally, we inhibited the growth of autophagy with 3-MA in parallel with the knockdown of Plin2. At that time, we found that the growth of autophagy-related proteins was significantly reduced. In addition, the increased HBV DNA replication was also reduced, and HBsAg and HBeAg were similarly altered. All these results indicate that the Plin2-autophagy pathway is involved in the regulation of high-fat inhibition of HBV replication.

CONCLUSION

In summary, our data suggest that fatty acid stimulation inhibits HBV replication by upregulating Plin2 expression, inhibiting hepatocyte autophagy. This process associates with lipid metabolism, autophagy pathway, and HBV replication. Further study of lipid metabolism-Plin2-autophagy is important to understand HBV host interactions and pathogenesis better and suggests a possible route for treating patients with chronic HBV infection combined with NAFLD.

ARTICLE HIGHLIGHTS

Research background

The relationship between lipid metabolism and hepatitis B virus (HBV) deoxyribonucleic acid (DNA) replication and its underlying mechanisms are not well understood.

Research motivation

To investigate the relationship between lipid metabolism and HBV DNA replication and its underlying mechanisms.

Research objectives

We speculated that the Plin2-autophagy pathway might be involved in regulating lipid deposition and HBV replication in hepatocytes.

Research methods

We first explored the relationship between patients' lipid levels and HBV DNA load. Also, we constructed an HBV infection combined with a hepatic steatosis cell model *in vitro*.

Research results

Stratified analysis by HBeAg showed significant negative correlations between HBV-DNA load and hepatic steatosis ratio in both HBeAg-positive group and in HBeAg-negative group. The results of *in vitro* experiments suggested that fatty acid treatment increased the lipid droplets deposition and decreased the cell supernatant HBsAg, HBeAg expression and HBV DNA load.

Research conclusions

Fatty acid stimulation inhibits HBV replication by upregulating Plin2 expression, inhibiting hepatocyte autophagy.

Research perspectives

A possible route for treating patients with chronic HBV infection combined with nonalcoholic fatty liver disease.

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FOOTNOTES

Co-first authors: Chuang Wang and Xiao-Yun Gao.

Co-corresponding authors: Chun-Wen Pu and Xuan Du.

Author contributions: Du X and Gao XY designed the experiment and drafted the manuscript; Wang C performed the experiments; Du X and Wang C participated in the statistical analyses; Han M, Shi XY, and Jiang CM helped draft the manuscript; All authors have read and approved the final manuscript.

Institutional review board statement: The study has been approved by the Ethics Committee of Dalian Sixth People's Hospital. The privacy rights of human subjects were always respected during human experimentation, and informed consent was obtained prior to the

experiment. The ethics program number is DLY/CB-IRB-026.

Institutional animal care and use committee statement: This study does not involve 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: All 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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