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EDITORIAL

Transplacental transmission of dengue infection

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Abstract

We specifically addressed the persistent challenge of dengue in endemic regions, highlighting the potential seriousness of dengue infection through vertical transmission. Vertical dengue transmission has been well documented, particularly in hyper-endemic regions, including Ecuador. Herein, we present a neonate diagnosed with congenital dengue and review similar cases from previously published reports. Although congenital dengue is commonly infected with severe serotypes of DENV (DENV-1 and DENV-2) infections, favorable outcomes are generally observed.

Key Words: Vertical transmission; Transplacental; Congenital dengue; Infectious disease; DENV

Core Tip: Dengue, caused by the dengue virus, poses a significant public health concern in tropical regions. While primarily known for its various clinical presentations, severe forms affecting pregnant women and children have been documented. Although vertical transmission of the virus from mother to fetus is uncommon, it can lead to severe complications, underscoring the need for vigilance and specialized care for both mothers and newborns.

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INTRODUCTION

Dengue is an emerging tropical disease caused by the dengue virus (DENV) with various clinical manifestations. Although the viral etiology of dengue fever (DF) was identified many years ago, DENV infection remains a public health problem, particularly in tropical regions[1]. Severe clinical forms of dengue infection have been well-documented in pregnant women and children[2]. Moreover, the incidence of DF in pregnant women is increasingly reported, and in several cases, DENV could be identified in the placenta[3], implying a possible intrauterine (vertical) transmission of dengue infection. Vertical transmission is not considered a common mode of dengue infection, and the transmission rate is likely quite low[4]; thus, the case has been infrequently reported globally. Nonetheless, it should be noted that mothers and newborns are prone to dengue shock syndrome and other bleeding manifestations, which require special attention.

VERTICAL DENGUE INFECTION AND SEVERE LIVER FAILURE

A 17-year-old primigravid woman at 37 weeks of gestation was admitted to the Verdi Cevallos Balda public hospital complaining of fever, retro ocular headache, nausea, arthralgia, and myalgia. One day after the admission, patient progressed with edema in the lower limbs and vomiting. A complete laboratory examination revealed a leucocyte count of 8190/mm^3 (93.9% of neutrophils), hemoglobin of 10 g/dL, and thrombocyte of 265000/mm^3. Later, it is notable that she lived in a high-risk dengue area, the NS-1 antigen test was then evaluated, and DENV infection was confirmed. On the fifth day of hospitalization, epistaxis and rectal bleeding were noticed. And due to the progression of the disease, an emergency cesarean delivery was performed. Postoperatively, the patient was admitted to the intensive care unit for further observation and treated according to the current dengue management guidelines.

The neonate was a female, weighing 2640 g, with an APGAR score of 9 at the 5th minute of life, and she appeared well at birth. There was no history of mosquito bites after birth. A laboratory examination was conducted on the first day, resulting in negative findings for both NS-1 antigen and IgM anti-dengue virus. Other examinations were normal (Figure 1). However, she developed a fever (38.2 °C) on day 4 after birth, followed by jaundice in Kramer's zone 2 and an increase of bilirubin levels (Figure 1). Dengue infection was confirmed after re-assessment of NS-1 antigen. Patient was then transferred to the neonatal intensive care unit for further evaluation. The complete blood count was checked regularly. Her thrombocyte count reduced significantly, reaching the lowest value of 11000/mm^3; thrombocyte transfusion was then initiated (Figure 1). Hemoglobin and leucocyte were within a normal limit throughout the illness. The complete blood count variation is depicted in Figure 1. The neonate was finally diagnosed with congenital dengue and neonatal jaundice. Such findings indicated a potential risk of acute liver failure, especially in endemic regions, which is in line with the study by Singh *et al*[5]. She and her mother were treated according to the dengue management guidelines. Her treatment also included thrombocyte transfusion, anti-inflammatory and antipyretic drugs. She was discharged from the hospital on day 19 in stable condition. Her mother is responsive to the treatment, with no complications, stable within two weeks with normal white cell and platelet counts, and discharged on day 15.

The hyper-endemicity of dengue in several countries, including Ecuador, poses a potential threat to pregnant women and possible vertical transmission to new babies. The incidence of congenital dengue is rare; nevertheless, clinical manifestations and severity between cases vary depending on the newborn's condition. Similar to our cases, congenital dengue is usually manageable with appropriate treatment, and patients are discharged within 1–8 weeks. In order to understand and synthesize a comprehensive view of the case, we also conducted a literature review presenting similar cases by querying from the following database: PubMed, Scopus, and Google Scholar, with the keyword such as "vertical dengue infection", "transplacental dengue infection", "neonate", and "dengue", without any restriction on language and year of publication. After removing some duplications, we ended up with 50 publications related to our case from various regions, including South East Asia[3,6-14], South Asia[15-24], Central Asia and the Middle East[25-34], South America[35-44], and other regions[45-54]. Nonetheless, it is worth noting that among 154 previously reported cases, 12 cases (7.8%, Figure 2)[3,20,22,24,29,30,35,37] died due to several complications and secondary illnesses, including heart anomalies, low birth weight, and sepsis. Although in most cases, maternal outcome among newborns who died is generally good and discharged within a week, some died due to severe forms of dengue and possibly associated with hypoxia[3,20]. In principle, treatments of congenital dengue are similar among reported cases. Interestingly, a sudden drop in thrombocyte level was observed after a patient received thrombocyte concentrate. At first, we suspected that patient had a refractory

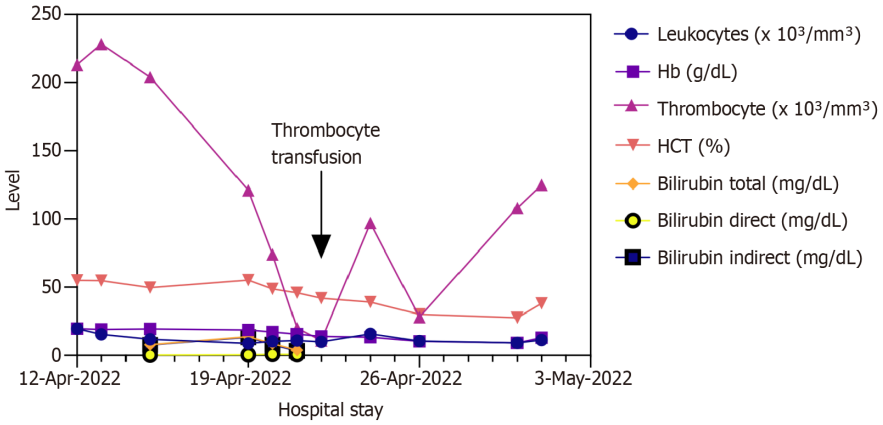

Figure 1 Complete blood count and bilirubin evaluation throughout the illness.

Country	No. cases	DENV type	Outcomes	References
Ecuador	2	-	Discharged	This case; Suarez, 2021
Indonesia	1	DENV2	Discharged	Haryanto, 2019
China	1	DENV1	Discharged	Yin, 2016
Thailand	13	DENV1, DENV2, DENV4	Discharged	Petdachai, 2004; Sirinavin 2004; Witayathawornwong, 2012; Janjindamai and Pruekprasert, 2003; Kerdpanich, 2001; Phongsamart, 2008; Chotigeat, 2003; Aurpibul, 2014; Thaithumyanon, 1993
Colombia	22	-	No information	Restrepo, 2004
Saudi Arabia	1	-	Discharged	Alallah, 2019
Brazil	33	DENV2	7 Died 26 Discharged	Maroun, 2008; Nunez, 2017; Riberio, 2013; Riberio, 2017
Vietnam	31	-	Discharged	Nguyen, 2021
India	17	-	3 Died 12 Discharged	Gupta, 2020; Swaminathan, 2019; Kaur, 2014; Madireddi, 2021; Inamdar and Danish, 2018; Pothapregada, 2017; Kaur, 2020; Mounica, 2021; Choudry, 2004; Chopra, 2013; Razak, 2015; Krithika, 2016
Sri Langka	6	-	2 Died 4 Discharged	Sinhabahu, 2014; Bopeththa, 2018; Ekanayake, 2014; Mehndiratta, 2016
Malaysia	4	DENV2	Discharged	Chye, 1997; Chin, 2008; Pitchaimuthu, 2021;
Bangladesh	1	-	-	Fatimil, 2003
Pakisatan	1	-	Discharged	Shabbir and Ehsan, 2021
Puerto Rico	1	DENV1	Discharged	Pérez-Padilla, 2011
Argentina	1	DENV1	Discharged	Berberian, 2011
Boliva	1	DENV2	Discharged	Aburdene, 2012
Peru	1	DENV2	Discharged	Arteaga-Livias, 2017; Silva Delgado, 2011
Mexico	10	DENV1	Discharged	Castellanos-Morfín, 2006; Ramírez, 2014; Romero-Santacruz, 2015; Morgan-Ortiz, 2014
Taiwan	1	-	Discharged	Yang,2015
France polinesia	6	-	Discharged	Mazarin, 2014; Boussemart, 2001

Figure 2 Vertical transmission of dengue infection in previously reported studies.

state of thrombocytopenia. However, thrombocyte levels slowly increased over time and thus reflecting a reduction of vascular permeability and an improvement in patient outcomes.

Although dengue serotype was not determined in this case, DENV-4 is typically prevalent in Ecuador, Venezuela, Colombia, and Brazil[55]. Moreover, a study indicates that the DENV-1 and DENV-2 serotypes are closely linked with dengue severity, marked by low antibody levels, compared to DENV-4-infected patients[56,57]. Indeed, DENV-1 and DENV-2 serotypes are commonly identified in congenital dengue (Figure 2). These results imply that vertical transmission of DENV may occur when mother had a low antibody response against dengue infection, particularly due to DENV-1 and DENV-2 serotypes. The placenta is an organ that can effectively reflect the inflammatory response, virus presence, and maternal hemodynamic alterations[3]. As such, examining the immunolocalization of anti-DENV complex in the placenta can be a reliable method for diagnosing maternal dengue. This approach is particularly advantageous since placenta samples are easily accessible for analysis.

CONCLUSION

In summary, although identification of DENV serotypes may not influence dengue management, early detection of serotypes circulating in the territory may help to prevent severe dengue cases in pregnant women and subsequently prevent possible cases of congenital dengue during dengue outbreaks.

FOOTNOTES

Author contributions: Zambrano LEA, Zevallos VMV conceived the report and data collection; Zambrano LEA is the main contributor to this article; Soraya GV, Istifiani LA, Pamungkas SA performed a literature review; Ulhaq ZS conceived the report, performed a literature review, and wrote the article.

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OPINION REVIEW

Resurgence of dengue in the Philippines

Jasmine S Interior, Kyrsten Jannae J Bigay, Remigo Angelo A Iringan, Mary Beth F Tanco

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic has significantly influenced the epidemiological landscape of various infectious diseases such as dengue. Dengue is an endemic disease in the Philippines, which showed a significant decline in the number of cases beginning in March 2020 due to the stringent public health measures implemented to curb COVID-19 cases. However, the easing of these restrictions subsequently led to a resurgence in dengue cases, as reported by the World Health Organization, with a notable increase compared to previous years. As the country navigates towards a post-pandemic phase, addressing the resurgence of dengue requires sustained efforts in vector control, surveillance, and healthcare preparedness. This article underscores the critical need for collaborative efforts among stakeholders to mitigate the resurgence of dengue while managing the ongoing recovery from the COVID-19 pandemic.

Key Words: Dengue; Dengue fever; COVID-19; Pandemic; SARS-CoV-2; Philippines

Core Tip: Maintaining robust public health measures implemented during the coronavirus disease pandemic and developing long-term strategies are crucial to mitigating the impact of dengue in the face of changing environmental and social dynamics. These measures not only address immediate outbreaks but also fortify the country's defenses against future infectious disease challenges as societal activities resume.

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INTRODUCTION

The societal disruption brought by the coronavirus disease 2019 (COVID-19) pandemic indirectly affected the dynamics of various infectious diseases[1]. Dengue fever, considered a major public health problem in tropical countries such as the Philippines[2], is one of the diseases found to be significantly affected[1]. The decline in the incidence of dengue from March 2020 was strongly associated with public health and social measures implemented by governments to limit the spread of COVID-19[1]. These include limitations of social gatherings, cancellation of face-to-face classes, increased work-from-home setups, and community lockdowns. However, the recent lifting of lockdown restrictions and the gradual increase in human mobility resulted in a 191% increase in dengue cases in the Philippines[3].

Philippines against dengue

One of the Philippine government programs is the National Dengue Prevention and Control Program. This aims to eliminate dengue infection by properly implementing an integrated vector control approach and simultaneously reinforcing the diagnosis, management, and surveillance of dengue cases[4]. The 5S strategy, the 4 o'clock habit, and the dengue fast lane are the three main campaigns of the Department of Health to prevent dengue. The 5S strategy encourages communities to search and destroy breeding sites, employ self-protection measures, seek early consultation, support fogging in hotspot areas, and sustain adequate hydration. The 4 o'clock habit specifically refers to a stop, look, and listen approach, whereby one drops whatever is being done to search and destroy mosquito breeding sites. Dengue fast lanes in hospitals are put in place to ensure that suspected dengue patients are given immediate and proper medical intervention.

The government also introduced the Dengvaxia vaccine through mass immunization campaigns in April 2016 to protect children from hospitalization and severe dengue. However, these efforts turned out to be futile as new findings revealed that the dengue vaccine could result in a higher risk of severe dengue infection to recipients without prior dengue infection. The Dengvaxia controversy spawned a significant decline in vaccine confidence in the country. Nevertheless, the culmination of efforts against dengue infection has resulted in a significant decline in dengue cases in the country; with cases dropping from 430282 in 2019 to 59675 in 2020 and mortalities dropping from 1612 in 2019 to 231 in 2020[5]. The Department of Health claims that current activities and initiatives against dengue, such as updating the Dengue Manual of Operations, partnering with tertiary hospitals, and releasing administrative issuance lowered dengue incidence and mortalities[5]. However, this must be taken with a grain of salt because the emergence of COVID-19 in 2020 also significantly contributed to the observed decline in dengue cases.

LIMITATIONS IN DENGUE RESPONSE IN THE PHILIPPINES

Despite the progress reported by the Department of Health in terms of prevention and control of dengue during the COVID-19 pandemic, there is an alarming rise in dengue cases in the country at the moment. Its alarming resurgence uncovers the presence of various lapses and barriers to healthcare. While the COVID-19 pandemic laid bare the systemic healthcare inequities faced by millions of Filipinos, it has ironically concealed the omnipresent lapses in our dengue response. This is primarily due to the health and sociopolitical policies put in place to mitigate the spread of the COVID-19 pandemic. However, the improving pandemic response, the diminishing fears of COVID-19 infection, and our slow return to the old normal are slowly uncovering the gaps in our dengue response, paving the way for its subsequent re-emergence in the Philippines.

CONCLUSION

Mitigating the resurgence of dengue relies heavily on controlling the vector that transmits it. The key to the decline of dengue morbidity and mortality can be achieved by properly implementing existing strategies and incorporating various modifications necessary as the country transitions to a new normal. These strategies include: (1) Proper implementation of the enhanced 5S-Strategy against dengue to protect the population from mosquito bites and prevent the spread of dengue; (2) Increase public awareness and education through health campaigns; (3) Strengthening of current surveillance and reporting systems to track outbreaks, monitor disease trends, evaluate progress in morbidity and mortality reduction goals, and consequently guide decision-making for quicker responses and better resource allocation; (4) Promotion of vaccine confidence through a coordinated, transparent, evidence-based education, and behavioral intervention campaign; (5) Allocation of health resources to cheap and simple dengue diagnostics like nucleic acid amplification test-loop mediated isothermal amplification assay; (6) Supporting research and projects like the Wolbachia Project within Bicol-Center for Health Development to control dengue spread; and (7) Increasing capacity building by training healthcare providers, ensuring adequacy of medical supplies, and improving healthcare facilities' capacity to diagnose and treat dengue promptly.

While we are recovering from the COVID-19 pandemic, we urge the government and relevant stakeholders to act hand in hand in transitioning to a new normal, without sacrificing all the progress that has already been made concerning dengue control and eradication.

FOOTNOTES

Author contributions: Interior JS conceptualized the study topic, formulated the questions to be answered by the study, did a comprehensive literature search, synthesized the data extracted, drafted the manuscript, critically reviewed the manuscript, and provided professional recommendations; Bigay KJJ conducted a comprehensive literature search, synthesized the data extracted, drafted the manuscript, ensured the accuracy and completeness of the review, and provided professional recommendations; Iringan RAA conducted a comprehensive literature search and helped draft the manuscript; Tanco MBF provided overall supervision and professional insights; All authors contributed to the final revision and approved the manuscript for publication.

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REVIEW

Role of vitamin D in COVID-19 and other viral infections

Muhammet Mesut Nezir Engin, Öner Özdemir

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Abstract

Vitamin D is a steroid hormone that is naturally produced in the body or obtained through dietary sources, primarily under the influence of UVB radiation. This essential nutrient has a vital role in numerous physiological processes, encompassing immune function, cell growth, differentiation, insulin regulation, and cardiovascular well-being, along with its pivotal role in sustaining the delicate equilibrium of calcium and phosphate concentrations in the body. Moreover, vitamin D reinforces mucosal defense and bolsters the immune system through immunomodulation, making it a critical component of overall health. Numerous studies have unveiled the profound connection between vitamin D and the predisposition to respiratory tract infections, including well-known viruses such as influenza and the novel severe acute respiratory syndrome coronavirus 2. Vitamin D deficiency has been consistently linked to increased severity of coronavirus disease 2019 (COVID-19) and a heightened risk of mortality among afflicted individuals. Retrospective observational studies have further substantiated these findings, indicating that levels of vitamin D are linked with both the occurrence and severity of COVID-19 cases. Vitamin D has its influence on viral infections through a multitude of mechanisms, such as promoting the release of antimicrobial peptides and fine-tuning the responses of the immune system. Additionally, vitamin D is intertwined with the intricate network of the renin–angiotensin system, suggesting a potential impact on the development of complications related to COVID-19. While further clinical trials and extensive research are warranted, the existing body of evidence strongly hints at the possible use of vitamin D as a valuable tool in the prophylaxis and management of COVID-19 and other viral infectious diseases.

Key Words: COVID-19; SARS-CoV-2; Vitamin D; Influenza virus; Viral infections

Core Tip: While further clinical trials and extensive research are warranted, the existing body of evidence strongly hints at the possible use of vitamin D as a valuable tool in the prophylaxis and management of coronavirus disease 2019 and other viral infectious disease.

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INTRODUCTION

Unlike other vitamins, vitamin D is a hormone that can be produced by the body by the action of UVB radiation on the skin or can be obtained from fish, milk, cereal products, and dietary supplements[1]. Vitamin D has a familiar role in maintaining calcium and phosphate balance, but recent research has revealed that it also has a role in immune function, cell proliferation and differentiation, insulin release, and cardiovascular health[2-4]. Vitamin D enhances mucosal defense by increasing immunity by secreting antiviral peptides with its immunomodulatory role[5,6]. Nutritional elements, such as vitamin D, known for its pivotal role in immune function, emerge as key players in this context. Recent data has shown the antiviral properties of vitamin D, capable of directly inhibiting viral replication, while also operating in an anti-inflammatory and immunomodulatory capacity.

Studies have linked vitamin D deficiency to acute respiratory infections with viruses such as influenza and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[7-9]. A meta-analysis conducted in 2019 examining the relation between vitamin D deficiency and the incidence of community-acquired pneumonia revealed that individuals with serum vitamin D concentrations < 50 nmol/L (equivalent to < 20 ng/mL) faced a 64% elevated risk of developing pneumonia[10]. It is also predicted that vitamin D deficiency may endanger the function of the pulmonary immune system and increase the risk of coronavirus disease 2019 (COVID-19) severity and fatality[11]. Understanding the alterations in the severity and mortality of COVID-19 is crucial, with a strong emphasis on enhancing nutrition and fortifying the immune system.

Carpagnano *et al*[12] identified a significant prevalence of vitamin D deficiency among COVID-19 patients experiencing acute respiratory failure. It is essential to recognize that vitamin D deficiency is connected to a range of health conditions and diseases that elevate the long-term susceptibility to contracting COVID-19. There is also a suggestion that vitamin D deficiency may promote long-term complications following COVID-19, and vitamin D use could potentially have a role in treatment. Nevertheless, further research is imperative in this area.

To examine the role of vitamin D in the severity and fatality of COVID-19 and other viral infections, a comprehensive review of existing studies was carried out. Databases such as Scopus, Google Scholar, Web of Science, PubMed, Cochrane Central Register of Controlled Trials, and medRXiv were systematically investigated for pertinent literature, encompassing discussions on the function, severity, and fatality aspects of vitamin D in viral infections[1-6].

METABOLISM OF VITAMIN D AND ITS DEFICIENCY

For a considerable duration, vitamin D was primarily recognized as a nutritional element integral to bone metabolism. Yet, modern insights have redefined its categorization as a steroid hormone, exposing its crucial regulatory functions within diverse physiological systems and pathways inherent to the body. Newly emerging evidence highlights the connection between vitamin D deficiency and various infectious diseases, particularly noteworthy when inadequate responses to standard treatments coincide with viral infections. Numerous clinical trials have illuminated the relationship between vitamin D deficiency and an elevated vulnerability to pulmonary infections. This connection is further substantiated by a multitude of laboratory experiments that emphasize the inhibitory role of vitamin D within the renin-angiotensin signaling pathway. The synthesis of vitamin D occurs in the dermis, initiated by the action of ultraviolet radiation on 7-dehydrocholesterol. Following this, vitamin D is conveyed to the liver, where it associates with the vitamin D binding protein and undergoes a transformation into its primary circulating form, 25-hydroxycholecalciferol (25(OH)D), facilitated by at least one cytochrome P450 (CYP) hydroxylase enzyme. Subsequently, the converted 25(OH)D travels to the kidneys, where an additional CYP hydroxylase enzyme initiates the synthesis of its hormonally active form, 1,25-dihydroxycholecalciferol (1,25(OH)2D3). However, despite the widespread recognition of the importance of vitamin D, achieving optimal levels remains a challenging endeavor for many individuals across the globe. vitamin D deficiency is a pervasive universal health fear, affecting an estimated population of > 3 billion people, with half of these individuals experiencing a genuine and clinically significant vitamin D deficiency[7,13-21] (Table 1).

Epidemiological studies consistently indicate a link between diminished plasma vitamin D levels and increased susceptibility to both acquiring and suffering from severe respiratory viral infections. These findings strongly indicate the potential utility of vitamin D intake in the realms of viral respiratory infection prevention and treatment. A meta-analysis

Table 1 Effects of vitamin D supplementation on the immune system and other different conditions[25]

Immune system component	Effect
T cells	↓ Th 1/Th 17 and ↑ Th 2
	↓ IL-8, IFN-γ, IL-12, IL-6, TNF-α, IL-17
	↑ IL-4, IL-5, IL-10
	Recognition of viral dsRNA by TLR - 3
B cells	↑ Apoptosis
Plasma cells	↓ Proliferation and immunoglobulin secretion
Neutrophils, monocyte-macrophages and dendritic cells	Reception to infectious areas, ↑ TLR
	↑ Intracellular killing of *Mycobacterium tuberculosis* (macrophages)
Infected cells	↑ Autophagy and apoptosis
Antimicrobial peptides (human cathelicidin peptide LL - 37 and β-defensin)	Augmented
Respiratory tract infections	Effect
Acute respiratory infections	↓ Proinflammatory cytokines in the lung through modulation of the activity of both macrophages and T lymphocytes
	↓ Risk of getting sick
VAP	↓ IL-6
	↓ Mortality rate
Autoimmune disease	Effect on disease
Type 1 diabetes	Prevention of onset, ↓ serum antibody levels, delayed β cell destruction in early stages of disease
Multiple sclerosis	Prevention of the onset
Rheumatic joint inflammation	Prevention of onset, reduced disease activity
Systemic lupus erythematosus	Prevention of onset, reduced disease activity
Crohn's disease	Prevention of the onset
Thyroiditis	Prevention of the onset
Psoriasis	Prevention of the onset
Polymyalgia rheumatica	Prevention of the onset
Autoimmune gastritis	Prevention of the onset
Systemic sclerosis	Downregulation of TGF-β/Smad signaling (putative antifibrotic effect in early stages of disease)
Pulmonary fibrosis	Effect
IL-1β	Decreased antagonism of pulmonary fibroblast cell activity in a murine model of bleomycin-induced lung fibrosis
Hydroxyproline, col1a1, col3a1, and α-smooth muscle actin mRNAs	Prevention of bleomycin-induced lung fibrosis in a mouse model

Th: T helper; IL: Interleukin; IFN: Interferon; TNF: Tumor necrosis factor; TLR: Toll-like receptor; VAP: Ventilator associated pneumonia.

revealed a reciprocal correlation between decreased serum 25(OH)D concentration and respiratory tract infections. According to this analysis, lower vitamin D levels were observed to be more efficacious in guarding against respiratory infections. Yet another meta-analysis identified a negative correlation between levels of circulating 25(OH)D and the risk as well as the severity of respiratory tract infections. Subjects with the lowest 25(OH)D concentrations < 15 ng/mL were determined to have the highest risk of respiratory tract infection[22-24].

The metabolism of vitamin D can be summarized as follows. In part A, how vitamin D regulates the immune system is discussed. In this section, vitamin D is specifically focused on T cells. The activity of T helper (Th)1 and Th17 cells is downregulated by vitamin D, while Th2 cells affect cytokine production. Proinflammatory type 1 cytokines [*e.g.*, interleukin (IL)-6, IL-8, IL-12, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and IL-17] decrease, while type 2 anti-inflammatory cytokines (*e.g.*, IL-4, IL-5, and IL-10) increase. Vitamin D has a function in regulation of the recognition of

viral double-stranded RNA (dsRNA) through Toll-like receptor 3. This regulatory function is pivotal in the immune response against viral infections. Vitamin D has a proapoptotic role by reducing the lifespan of B cells, the proliferation of plasma cells, and immunoglobulin production. Infected areas trigger the recruitment of neutrophils, monocytes, macrophages, and dendritic cells, which in turn enhance their intracellular pathogen-killing capabilities. This heightened immune response is especially critical in combating infections by pathogens such as *Mycobacterium tuberculosis*. Infected cells become more prone to autophagy and apoptosis. Finally, vitamin D may augment the production of antimicrobial peptides such as human cathelicidin peptide LL - 37 and β-defensin[25].

Part B deals with the influence of vitamin D on antiretroviral therapy. Vitamin D can mitigate the risk of disease development by participating in the reduction of proinflammatory cytokines. However, no clear effect on childhood pneumonia has been demonstrated. Decreased IL-6 levels and mortality rates have been reported in patients with ventilator-associated pneumonia (Table 1).

EFFECT OF VITAMIN D ON THE RENIN–ANGIOTENSIN SYSTEM

Vitamin D shows its effectiveness on the renin–angiotensin system (RAS) by inhibiting renin synthesis, reducing angiotensin-converting enzyme (ACE) and angiotensin (Ang) II production, and increasing ACE2/Ang-(1–7) axis activity. The active form of vitamin D (1,25(OH)2D3) directly inhibits renin synthesis. This effect is independent of Ang II feedback regulation. In *in vitro* studies, in cell lines with high renin expression levels, 1,25(OH)2D3 directly and comprehensively inhibits renin gene transcription. Vitamin D binds to vitamin D receptors (VDRs) in cells. This complex functions as a transcription factor that inhibits transcription of the renin gene. Binding of VDR to the promoter region of the renin gene directly reduces renin mRNA synthesis. 1,25(OH)2D3 supports the negative feedback mechanism on the RAS by reducing renin synthesis. This leads to lower levels of renin and Ang II, which in turn lowers blood pressure. Vitamin D may indirectly inhibit renin synthesis in the kidneys by reducing the production of inflammatory cytokines. Since inflammation is a factor that increases renin production, the anti-inflammatory effects of vitamin D may suppress renin synthesis[26,27].

In experimental studies conducted in rat models, the active form of vitamin D inhibits the production of renin, ACE and Ang II, while increasing the expression of ACE2. The active form of vitamin D directly inhibits ACE gene expression. This effect leads to decreased ACE levels and therefore decreased Ang II production. Vitamin D indirectly reduces Ang II production *via* ACE by reducing renin production. Decreased renin levels lead to decreased Ang I, which in turn leads to decreased ACE activity and Ang II production. Vitamin D may contribute to indirect inhibition of ACE and Ang II production by reducing the production of inflammatory cytokines. Inflammation is a factor that increases ACE and Ang II production. Vitamin D negatively regulates RAS by increasing the activity of the ACE2/Ang-(1–7) axis. This regulation provides a protective effect against acute lung injury[26-29].

BRIEF PATHOLOGY OF COVID-19

Chen *et al*[30] conducted a study using bronchoalveolar lavage samples from two patients exhibiting typical symptoms of COVID-19. They sequenced the complete genome of the SARS-CoV-2, comprising 29 881 nucleotides, utilizing a low-input metagenomic next-generation sequencing method. Their analysis unveiled a substantial 94.6% genetic resemblance between SARS-CoV-2 and SARS-CoV, suggesting their shared species origin. In previous years, it was established that SARS-CoV utilizes ACE2 as its primary receptor to infect host cells[26]. Building on this knowledge, Zhou *et al*[31] showed that SARS-CoV-2 similarly uses ACE2 as a cellular binding receptor in ACE2-expressing cells from humans, civets, pigs, and Chinese horseshoe bats (although not in mice). ACE2 acts as the cell fusion receptor, and its distribution in organs *e.g.* endothelium, lung, heart, kidney, and intestine aligns with the tropism of SARS-CoV-2. Preliminary investigations have associated SARS-CoV-2 infection with significant clinical manifestations, including acute respiratory distress, acute heart injury, acute renal failure, and gastrointestinal symptoms. ACE2, apart from its role in viral entry, serves as a second ACE, playing a vital role in regulating the RAS. The RAS system comprises numerous enzymes, peptides, and receptors critical for diverse biological functions like blood pressure and fluid balance in the body. Distinct from other RAS components such as ACE and Ang II, ACE2 has a protective role in mitigating lung injury. It counterbalances the effects of Ang II by converting it into Ang-(1–7), a peptide formed through ACE hydrolysis. Studies have indicated a connection between heightened ACE activity, linked to a deletion polymorphism in the ACE gene, and the development of ARDS and related fatality. Moreover, ACE2 has been shown to offer protection against sepsis-induced lung injury in mouse models. Therefore, ACE2 appears to be a pivotal factor both in facilitating the cellular entrance of SARS-CoV-2 and influencing the pathogenicity of the infection[30-38].

CURRENT STUDIES ON THE ROLE AND CONSEQUENCES OF VITAMIN D IN SARS-COV-2 INFECTION

Numerous retrospective observational studies have been undertaken to investigate the link between vitamin D levels and SARS-CoV-2 infection. Several of these studies have revealed substantial associations between vitamin D levels and the prevalence and severity of COVID-19. For instance, a study conducted in South Asian countries disclosed notable differences in vitamin D levels among mild, moderate, severe, and critical COVID-19 cases. Similarly, a cohort study in

Singapore demonstrated that patients receiving vitamin D, magnesium, and vitamin B12 supplements needed less oxygen therapy and exhibited protective effects against clinical deterioration. Other investigations have reported reduced vitamin D levels in cases with severe COVID-19 and those with predisposing medical disorders. A study from Belgium indicated that vitamin D levels in COVID-19 cases were meaningfully lower compared to a control group. Moreover, a study using data from diverse regions across the world suggested a 19% decrease in the number of severe COVID-19 cases in populations with normal vitamin D levels. A retrospective cohort study in Indonesia identified higher mortality rates among older COVID-19 male patients with low vitamin D levels and pre-existing medical conditions. Importantly, this study revealed a robust correlation between vitamin D levels and COVID-19 fatality, even after adjusting for potential confounding factors. A study done in the US mainland highlighted the link between sunlight exposure, vitamin D levels, and reduced risk of COVID-19 cases and mortality. While several observational research has indicated associations between vitamin D concentrations and COVID-19 incidence and severity, it is crucial to acknowledge that the results across these studies are not always consistent. A shortage of clinical trials and cohort research hinders the establishment of conclusive evidence concerning the function of vitamin D in the prophylaxis or management of COVID-19 (Table 2). Consequently, additional research is essential to provide more definitive insights into this matter[31-33,39-42].

POTENTIAL EFFECTS OF VITAMIN D ON COVID-19 COMPLICATIONS

Vitamin D may play an important role in the management of COVID-19 complications through its regulatory effects on the RAS. By reducing the production of renin, ACE, and Ang II and increasing the expression of ACE2, vitamin D may reduce the risk of ARDS, inflammation, vascular damage, and thrombosis. These mechanisms suggest that vitamin D may be used as a potential adjuvant in the treatment of COVID-19 (Table 2). However, more clinical research is needed to better understand these effects[25-28].

EVALUATION OF FINDINGS IN THE CONTEXT OF AGE, GENDER AND SYSTEMIC CONDITIONS

In the studies presented in Table 3, we addressed the effects of vitamin D deficiency and supplementation on viral infections in the context of various age groups, genders, and pre-existing systemic conditions[43-50].

Vitamin D deficiency increases susceptibility to respiratory infections. Adequate vitamin D levels may play an important role in reducing the risk of acute respiratory infections. It appears that vitamin D supplementation may reduce the incidence of respiratory infections, especially in at-risk groups such as children, the elderly, and individuals with chronic diseases.

Studies in children and adolescents reveal that vitamin D deficiency increases the risk of upper and lower respiratory tract infections. It is stated that vitamin D supplementation is effective in reducing the incidence of viral infections such as influenza. A significant association has been found between low vitamin D levels and an increased risk of respiratory infections in adults and elderly individuals. The protective effects of vitamin D supplementation were also supported in this group.

Although the studies generally included both genders, no significant difference was observed between genders. In both men and women, adequate vitamin D levels appear to have similar effects in reducing the risk of respiratory infections.

In individuals with chronic respiratory diseases such as asthma and chronic obstructive pulmonary disease, vitamin D deficiency can lead to more frequent respiratory infections. It is stated that vitamin D supplementation can reduce the frequency of infections in these individuals. Similar findings have been obtained in individuals with chronic diseases such as diabetes, hypertension and HIV infection. While vitamin D deficiency may increase the risk of respiratory infections in these individuals, it appears that this risk can be reduced with supplementation[45-54].

On the contrary, Murdoch *et al*[48] and Belderbos *et al*[55] show that the protective effect of vitamin D supplementation in all populations is not always clear. For example, Murdoch *et al*[48] found that vitamin D supplementation had no protective effect against upper respiratory tract infections in healthy adults. McNally *et al*'s study, conducted in children in intensive care, emphasized that vitamin D deficiency is associated with infections and the importance of improving vitamin D levels in these children[56].

As a result, vitamin D deficiency stands out as a factor that generally increases the risk of respiratory tract infections. It is supported by many studies that vitamin D supplementation may be effective in reducing the incidence of infection, especially in at-risk groups. However, some studies also show that protective effects are not always consistent, so more research is needed to better understand the effects of vitamin D supplementation.

CLINICAL EFFECTS OF VITAMIN D SUPPLEMENTATION ON VIRAL INFECTIONS AND DOSAGE CONTROVERSIES

The overall findings of the studies in Table 4 reveal the negative effects of vitamin D deficiency on viral infections, especially COVID-19. It is suggested that vitamin D supplementation may reduce the severity of infections and mortality, especially in high-risk groups (older people, those with chronic diseases). However, some studies also show that the effect of high-dose vitamin D supplementation on clinical outcomes is limited. Therefore, more research is needed to

Table 2 Correlation of vitamin D concentrations with severe acute respiratory syndrome coronavirus 2 infections and outcomes

Ref.	*n*	Population type	Study type	Vitamin D dosages	Results
Lau *et al*[111], 2020	20	Adults, average age 65.2 yr	Retrospective observational study	NA	Higher levels of vitamin D deficiency were observed in ICU patients (84.6%) compared to baseline patients (57.1%) (P = 0.29)
Hastie *et al*[115], 2020	449	Adults, age 37–73 yr	Cross-sectional study	NA	Vitamin D levels showed a significant association with SARS-CoV-2 infection in univariate analysis (P = 0.013)
Ilie *et al*[116], 2020	Cases and deaths/1 M population	Adults	Retrospective	NA	Negative correlation was observed between mean levels of vitamin D and COVID-19 cases (P = 0.050) and deaths (P = 0.053) per million population
Glicio *et al*[117], 2020	176	Adults, age ≥ 60 yr	Retrospective	NA	Severe patients are more likely than mild patients had a lower level of vitamin D
Tan *et al*[69], 2020	43	Adults, age ≥ 50 yr	Cohort observational	Vitamin D 1000 IU	Patients treated with vitamin D showed a significant protective effect against clinical deterioration after adjusting for age, sex and comorbidities (P = 0.041)
Darling *et al* [118], 2020	580 cases and 723 controls	Adults, average age 57.7 yr	Retrospective	NA	No significant difference was observed in vitamin D levels between COVID-19 cases and the control group
Raharusun *et al* [119], 2020	780 cases	Adults, average age 54.5 yr	Retrospective cohort study	NA	In univariate analysis, older and male cases with pre-existing medical conditions and below normal vitamin D levels were associated with higher mortality rates
Daneshkhah *et al* [120], 2020	5000 cases	Age ≤ 80 yr	As of March 21, 2020	NA	Approximately 15% reduction in the number of severe COVID-19 cases was observed in a population given a normal vitamin D status

NA: Not available; ICU: Intensive care unit; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019.

better understand the effects of vitamin D supplementation and determine optimal dosage[57-66].

VITAMIN D AND MECHANISMS TO REDUCE VIRAL INFECTIONS

Most effects of vitamin D are intricately tied to the recruitment of calcitriol to the nuclear VDR. This receptor, located within the cell nucleus, orchestrates the assembly of active chromatin complexes that, in turn, instigate genetic and epigenetic modifications of transcriptional output. This occurs by the direct interaction of the receptor with regulatory sequences situated in proximity to the target genes. One of the most well-known functions of calcitriol, the active vitamin D, is its role in regulating serum calcium levels, forming a feedback loop with parathyroid hormone (PTH). In this intricate interaction, vitamin D ensures the harmonious absorption and utilization of calcium by counteracting the effects of PTH, ultimately serving to keep calcium concentration within a narrow range. Along with its role in calcium regulation, vitamin D boasts a plethora of other effects. It has a vital part in managing calcium and phosphate metabolism, overseeing the mineralization of bone, regulating the working of the immune system, and controlling the processes of cellular growth and differentiation. Through its genetic and epigenetic actions, vitamin D yields a far-reaching impact on health, influencing a wide array of biochemical and physiological processes within cells. This underscores the multifaceted role of vitamin D in sustaining overall healthiness[67-71].

Many studies have examined the mechanisms by which vitamin D reduces the risk of viral infections (Table 3). Vitamin D uses a variety of mechanisms to reduce the risk of microbial infections and associated mortality. A current review has categorized the role of vitamin D in reducing the risk of viral infections into three key categories.

Physical barrier enhancement

Vitamin D contributes to the maintenance of robust physical barriers that prevent the invasion of pathogens. It helps in preserving the integrity of tight junctions, gap junctions, and junctional complexes between cells, such as through E-cadherin. This maintenance of cell junctions acts as a protective physical barrier against infections. When these connections are compromised, it can elevate the risk of microbial invasion. Vitamin D has a function in preserving the integrity of these junctions, thereby reducing the susceptibility to infections.

Innate cellular immunity activation

Vitamin D is involved in the activation of the innate cellular immune system. It enhances the antiviral activity of cells, thereby providing a protective effect against various microbes. This aspect of vitamin D contributes to the body's initial defense against pathogens, including viruses.

Table 3 Effect of vitamin D levels on viral infections according to age, gender and systemic conditions

Ref.	Age group	Gender	Preexisting conditions	Results
Martineau *et al*[43], 2017	0–95 yr	Both genders	Asthma, COPD	Vitamin D supplementation is effective in reducing the risk of acute respiratory infections
Ginde *et al*[9], 2009	≥ 20 yr	Both genders	Chronic diseases (DM, HT)	Vitamin D deficiency is associated with the prevalence of upper respiratory tract infections
Sabetta *et al*[44], 2010	20–89 yr	Both genders	Chronic diseases	The risk of respiratory tract infection is reduced in individuals with serum 25(OH)D levels above 38 ng/mL
Cannell *et al*[7], 2006	0–90 yr	Both genders	Various health conditions	Vitamin D deficiency may increase susceptibility to influenza and respiratory infections
Laaksi *et al*[45], 2007	18–28 yr	Male	Healthy individuals	Vitamin D supplementation may reduce incidence of respiratory infections
Urashima *et al*[46], 2010	6–15 yr	Both genders	Healthy children	Vitamin D supplementation is effective in reducing the incidence of influenza A
Berry *et al*[47], 2011	≥ 65 yr	Both genders	Chronic diseases	Vitamin D deficiency is associated with risk of respiratory infections
Murdoch *et al*[48], 2012	50–84 yr	Both genders	Chronic diseases (COPD)	Vitamin D supplementation has no protective effect on respiratory infections
Jolliffe *et al*[49], 2020	0–95 yr	Both genders	Asthma, COPD	Vitamin D supplementation is effective in reducing the risk of acute respiratory infections
Camargo *et al*[50], 2012	3–24 yr	Both genders	Healthy children	Vitamin D deficiency may increase risk of acute lower respiratory tract infections
Hollams *et al*[51], 2011	0–10 yr	Both genders	Asthma, allergy	Vitamin D deficiency is associated with asthma and respiratory infections
Majak *et al*[52], 2011	5–18 yr	Both genders	Asthma	Vitamin D supplementation may reduce infection frequency in children with asthma
Esposito *et al*[53], 2013	0–16 yr	Both genders	Healthy children	Vitamin D deficiency may increase risk of respiratory infections
Thornton *et al*[54], 2014	18–45 yr	Both genders	HIV positive individuals	Vitamin D deficiency is associated with risk of respiratory infections
Belderbos *et al*[55], 2011	0–1 yr	Both genders	Healthy babies	Vitamin D deficiency may increase the risk of respiratory syncytial virus bronchiolitis
McNally *et al*[56], 2009	0–17 yr	Both genders	Chronic diseases	Vitamin D deficiency associated with respiratory tract infection in intensive care
Le Goaziou *et al* [57], 2011	0–16 yr	Both genders	Healthy children	Vitamin D deficiency is associated with risk of upper respiratory tract infections
Liu *et al*[58], 2020	0–18 yr	Both genders	Chronic diseases (asthma, COPD)	Vitamin D deficiency associated with risk of viral respiratory infections
Grant *et al*[59], 2009	0–95 yr	Both genders	Various health conditions	Vitamin D deficiency may increase risk of influenza and pneumonia
Aloia *et al*[60], 2007	18–45 yr	Both genders	HIV positive individuals	Vitamin D deficiency is associated with risk of respiratory infections

COPD: Chronic obstructive pulmonary disease; DM: Diabetes mellitus; HT: Hypertension.

Support for adaptive immunity

Vitamin D also has a critical function in the adaptive immune system. It supports the proper functioning of immune cells and regulates inflammatory responses. By doing so, vitamin D aids in controlling the spread of microbial infections and moderating the body's immune responses, preventing excessive inflammation and tissue damage.

In summary, the multifaceted role of vitamin D in bolstering physical barriers, activating the innate cellular immune system, and regulating the adaptive immune system collectively contributes to lessening the risk of microbial infections and their associated consequences, underscoring the importance of sustaining satisfactory vitamin D levels for general well-being and immune function[71-74].

Vitamin D has a pivotal role in enhancing cellular innate immunity through several mechanisms, including the stimulation of antimicrobial peptides like human cathelicidin (LL-37) and defensins. These antimicrobial peptides display direct antimicrobial activity against a broad spectrum of pathogens, including Gram-positive and Gram-negative bacteria, enveloped and nonenveloped viruses, and fungi. They accomplish this by disrupting the cell membranes of pathogens

Table 4 Summary of current studies examining the use of vitamin D in coronavirus disease 2019 and other viral infections conditions

Ref.	*n*	Vitamin D type	Vitamin D dosage	Application method	Viral infection	Disease status	Results
Entrenas Castillo *et al* [61], 2020	76	Vitamin D3 (calcifediol)	0.532 mg on day 1, then 0.266 mg on days 3 and 7, and weekly thereafter	Oral	SARS-CoV-2	Mild–moderate	The need for intensive care and the mortality rate were lower in patients receiving vitamin D treatment
Murai *et al* [62], 2021	240	Vitamin D3 (cholecalciferol)	200000 IU loading dose	Oral	SARS-CoV-2	Mild–moderate	High-dose vitamin D treatment did not improve clinical outcomes of COVID-19 patients
Rastogi *et al* [63], 2020	40	Vitamin D3 (cholecalciferol)	60000 IU/d for 7 d	Oral	SARS-CoV-2	Light	Vitamin D treatment shortened the time to PCR negativity
Maghbooli *et al*[64], 2020	235	Vitamin D3 (cholecalciferol)	50000 IU/wk	Oral	SARS-CoV-2	Mild–moderate	Adequate vitamin D levels shortened hospitalizations and reduced rates of serious illness
Annweiler *et al*[65], 2020	77	Vitamin D3 (cholecalciferol)	80000 IU single dose	Oral	SARS-CoV-2	Moderate–severe	COVID-19-related mortality rates were lower in patients receiving vitamin D therapy
Cangiano *et al* [66], 2020	90	Vitamin D3 (cholecalciferol)	25000 IU/mo	Oral	SARS-CoV-2	Moderate–severe	Severity of COVID-19 symptoms decreased in older individuals with vitamin D deficiency
Giannini *et al* [67], 2021	100	Vitamin D3 (cholecalciferol)	100000 IU/mo	Oral	SARS-CoV-2	Mild–moderate	High doses of vitamin D were found to be effective in reducing complications due to COVID-19
Ling *et al*[68], 2020	50	Vitamin D3 (cholecalciferol)	400 IU/d	Oral	Respiratory tract infections	Mild–moderate	Vitamin D supplementation has been found effective in reducing the incidence of respiratory infections
Tan *et al*[69], 2020	43	Vitamin D3 (cholecalciferol)	1000 IU/d	Oral	SARS-CoV-2	Moderate–severe	Vitamin D supplementation was found to be effective in reducing hospital stay and complications

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

and can nullify the biological activities of endotoxins. For instance, in mouse models, LL-37 has demonstrated the capacity to reduce the replication of influenza A virus. Moreover, 1,25-dihydroxyvitamin D, the active vitamin D form, has been shown to diminish rotavirus replication both *in vitro* and *in vivo*. A clinical trial described that 4000 IU/day of vitamin D reduced the incidence of dengue disease. Vitamin D also exerts an influence on cellular immunity by mitigating the cytokine storm triggered by the innate immune system. When the innate immune system responds to viral and bacterial infections, it produces a mix of proinflammatory and anti-inflammatory cytokines. Vitamin D can decrease the synthesis of proinflammatory Th1 cytokines, *e.g.* TNF-and IFN-γ. Additionally, vitamin D administration increases the expression of anti-inflammatory cytokines by macrophages while concurrently declining the expression of proinflammatory cytokines. The ability of vitamin D to stimulate antimicrobial peptides, dampen pathogen replication, and modulate the cytokine response serves as a multifaceted approach in bolstering the innate and cellular immune systems, ultimately contributing to a more balanced and effective immune response to infections[11]. These mechanisms are illustrated in Figure 1.

Vitamin D functions as a modulator of adaptive immunity, and its active form, 1,25-dihydroxy vitamin D3 (1,25(OH)2D3), has several effects on immune responses.

Th1 suppression

1,25(OH)2D3 can suppress Th1-mediated responses, specifically by reducing the formation of inflammatory cytokines like IL-2 and IFN-γ. This leads to a dampened Th1 response, which is recognized for its proinflammatory properties.

Th2 promotion

Conversely, 1,25(OH)2D3 helps cytokine making by Th2 cells, which tend to produce anti-inflammatory cytokines. This balance helps in moderating immune responses.

T regulatory cell induction

1,25(OH)2D3 also plays a role in the stimulation of T regulatory cells (Tregs), which are crucial for controlling immune responses and preventing excessive inflammation.

In the context of COVID-19, it is worth noting that serum 25(OH)D levels are inclined to wane with age. Since case–fatality rates for COVID-19 rise with age, this age-related decline in vitamin D levels may be of implication. Reduced

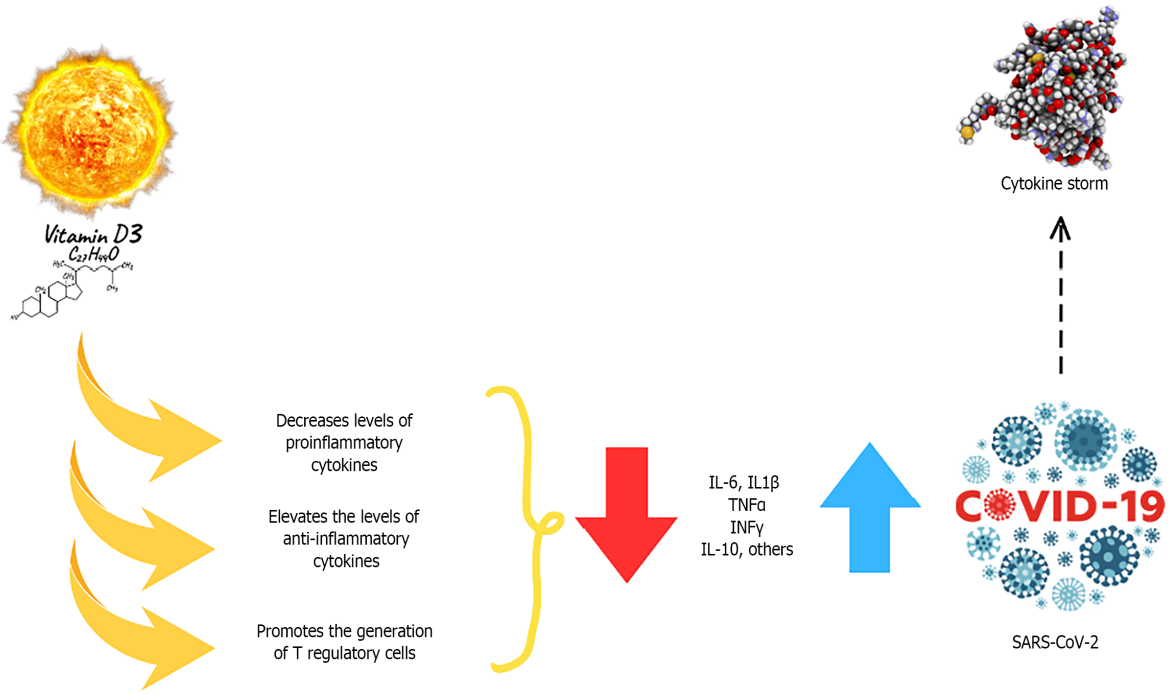

Figure 1 Mechanisms by which vitamin D may reduce the risk of cytokine storm. The blue up arrow indicates an increase and the red down arrow indicates a decrease. IL-6: Interleukin-6; IL-1β: Interleukin-1β; TNFα: Tumor necrosis factor α; INF-γ: Interferon γ; IL-10: Interleukin-10.

exposure to sunlight, often due to aging, can lead to decreased vitamin D production, as the skin has lower levels of 7-dehydrocholesterol, a precursor necessary for vitamin D synthesis. Furthermore, some pharmaceutical drugs stimulate the pregnane-X receptor and, as a side effect, reduce serum 25(OH)D levels. These drugs include antibiotics, anti-inflammatory agents, antihypertensives, antiretrovirals, antiepileptics, antineoplastics, endocrine, and certain herbal drugs. It is important to note that pharmaceutical drug use naturally surges with age, potentially compounding the age-related decline in vitamin D levels. Vitamin D supplementation has been related to the amplified expression of antioxidation-related genes, which in turn can support the action of other antioxidants like vitamin C (ascorbic acid). Vitamin C is recognized to possess antimicrobial activity and is recommended for preventing and treating COVID-19. Prominent health figures have also suggested the probable function of vitamin D in addressing the COVID-19 pandemic. However, it is essential to emphasize that while these connections are noteworthy, more comprehensive research and clinical trials are required to demonstrate the precise function of vitamin D in COVID-19 prevention and treatment[75-85].

POTENTIAL MECHANISMS OF VITAMIN D TO REDUCE COVID-19 PROGRESSION

Numerous clinical and epidemiological studies have provided compelling evidence for a significant interplay between vitamin D and the complex network of RAS. In experimental studies involving rats, the active vitamin D form has been demonstrated to inhibit the creation of renin, ACE, and Ang II. Conversely, it increases the expression of ACE2, particularly in the context of lipopolysaccharide-induced acute lung injury (ALI). In essence, vitamin D appears to enhance the activity of the ACE-2/Ang-(1–7) axis by negatively regulating the RAS while concurrently reducing the activities of the renin and ACE/Ang II pathway. This modulation has been observed to have protective effects in animal models, where blocking the (pro)renin receptor led to a reduced inflammatory response in pulmonary cells, offering protection against lipopolysaccharide-induced ALI. Vitamin D has demonstrated the capacity to suppress renin production, and this effect appears to be independent of Ang II feedback regulation. In mice, vitamin D deficiency causes increased renin synthesis, whereas supplementation with 1,25(OH)2D3 inhibits renin expression. Moreover, *in vitro* studies have revealed that in cell lines characterized by high renin expression levels, 1,25(OH)2D3 directly and comprehensively inhibits the transcription of the renin gene through a VDR-mediated mechanism. This intricate relationship between vitamin D and the components of the RAS has implications for various aspects of health, including susceptibility to respiratory infections. Emerging data suggests that different RAS components have a role in the progress of complications associated with COVID-19, underscoring the importance of understanding the regulatory interplay between vitamin D and these elements. While it is not yet fully elucidated how poor vitamin D concentration contributes to the progress and aggravations of viral diseases, several hypotheses have been proposed. Research continues to elucidate these connections, emphasizing the relevance of vitamin D in respiratory health and its potential impact on viral infections[86-92].

The VDR is expressed at high levels in many immune system cells, including dendritic cells, as well as T and B lymphocytes. Once vitamin D interacts with VDR, it performs as a transcription factor, modifying the responses of these immune cells to viral infections. Importantly, VDR is also expressed in pulmonary tissue, and its role in lung health is

evident. *In vivo* studies involving rodents and VDR-knockout mice have provided valuable insights. These studies have indicated that VDR-knockout mice experience more severe LPS-induced ALI with higher mortality rates. The deficiency of VDR in lung cells leads to increased expression of Ang II, heightened alveolar permeability, pulmonary vascular leakage, elevated neutrophil infiltration, enhanced apoptosis, heightened respiratory inflammation, and increased expression of proinflammatory cytokines and chemokines. Additionally, a specific VDR polymorphism known as the FokI T allele has been related to an amplified susceptibility to viral infections caused by enveloped viruses. The FokI polymorphism involves genetic variations that alter the interaction between VDR and transcription factors, resulting in functional modifications of VDR. This polymorphism can lead to changes in the transcriptional activity of VDR, which in turn can impact the response to viral infections. In summary, the presence and function of VDR in immune cells and lung tissue underscore the vital role of vitamin D in regulating immune reactions and maintaining respiratory health. Variations in the VDR gene, such as the FokI polymorphism, can further influence an individual's susceptibility to viral infections, especially those caused by enveloped viruses. Understanding these genetic and immunological aspects provides valuable insights into the complex interplay between vitamin D and the immune system[39,90-96].

The study conducted by Hansdottir and colleagues sheds light on the expression of key genes in primary lung epithelial cells, providing valuable insights into the role of vitamin D in immune regulation. These cells were found to express high levels of the CYP27B1 gene and low levels of the CYP24A1 gene[18,22].

CYP27B1 is an enzyme responsible for converting the circulating vitamin D form, 25(OH)D3, into its active hormonal form, 1,25(OH)2D3. In contrast, CYP24A1 degrades the active vitamin D form. This active vitamin D, 1,25(OH)2D3, plays a pivotal role in the regulation of the immune system. Crucially, immune cells can convert the inactive form of vitamin D into its active form, facilitated by the CYP27B1 enzyme. This conversion is closely associated with the making and regulation of antimicrobial peptides. Among these peptides, defensins hold a significant role. They are produced by the human airway epithelium and are found in the respiratory tract, where they function in defending the respiratory mucosa. When vitamin D is activated locally, it can straightly stimulate the expression of cathelicidin peptides, particularly cathelicidins. In the context of viral infections, vitamin D can synergistically interact with the active form of viral RNA, leading to intensification in the expression of antimicrobial peptides, notably cathelicidins. Cathelicidins are a group of peptides that are part of the innate immune system in various vertebrates, and they possess direct and indirect antimicrobial activity against a range of pathogens, comprising enveloped viruses. The vitamin D–cathelicidin axis plays a crucial function in the control of the human immune system, modulating both innate and adaptive immunity. One member of the cathelicidin family, LL-37, is produced by respiratory epithelial cells and enhances the ability to combat microbes, particularly respiratory pathogens. Vitamin D can trigger the expression of the LL-37 gene (CAMP), contributing to this antimicrobial activity. In summary, the study underscores the significance of vitamin D in the regulation of immune responses, particularly through its role in the induction of antimicrobial peptides like cathelicidins, which are instrumental in defending against a range of pathogens, containing respiratory viruses[15,97-111].

INFLUENZA AND VITAMIN D

Influenza virus exerts its effect on the respiratory tract by direct infection or by impairing the immune system reaction. Pneumonia usually develops due to influenza infection and is one of the causes of death. The risk of pneumonia is higher in groups such as individuals < 5 years of age, > 65 years of age, white individuals and those living in nursing homes, those with chronic lung or heart disease, smokers, and those with weakened immune systems. Seasonal influenza infections are generally more common during the winter months. This has been linked to the season when the sun's UVB rays, and therefore vitamin D (25(OH)D) levels, are lowest in most mid- and high-latitude countries during the winter months. Serum 25(OH)D levels are around 21 ng/mL in winter and 28 ng/mL in summer in the northern and central USA, and around 24 ng/mL in winter and 28 ng/mL in summer in southern regions. The winter peak also concurs with weather conditions such as low temperature and relative humidity that permit the influenza virus to endure longer. Ecological research shows that higher 25(OH) D levels with vitamin D use during the winter months may decrease the risk of catching influenza. Results of randomized controlled trials confirm that vitamin D intake has beneficial effects in lessening the risk of influenza. However, some of these studies included vaccinated participants or did not measure baseline 25(OH)D levels, which may affect the evaluation of results. Evidence on the effects of vitamin D on the immune system suggests that vitamin D supplementation may decrease the risk of flu, but more studies are needed. Additionally, large population research would be helpful to establish whether vitamin D utilization is accompanied by variations in serum 25(OH)D levels[102-110].

An observational study performed in Connecticut during the fall and winter of 2009–2010 studied the association between serum 25(OH)D concentration and the incidence of acute respiratory tract infections (ARTIs). In the study, 198 healthy adults were examined. During the study period, only 17% of subjects with 25(OH)D levels above 38 ng/mL developed ARTI, whereas 45% of subjects with 25(OH)D concentrations below 38 ng/mL developed ARTI. Levels of 38 ng/mL or higher were linked with a significant (P < 0.0001) twofold decrease in the risk of developing ARTI and a significant reduction in sick days. Eight influenza-like illnesses happened during this time, seven of which were caused by the 2009 H1N1 influenza virus[44].

Vitamin D metabolism and magnesium

Magnesium is required for vitamin D metabolism. Magnesium functions as a cofactor of enzymes critical for the production of calcitriol (1,25-dihydroxyvitamin D), the biologically active form of vitamin D. Magnesium deficiency can negatively affect vitamin D metabolism and therefore reduce the effectiveness of the immune system. Therefore, optimal

magnesium levels may increase the effectiveness of vitamin D supplements and strengthen immune resistance to infections. For example, in the Zittermann[112] study, it was shown that vitamin D supplements are more effective if magnesium is at sufficient levels.

SUN EXPOSURE AND VITAMIN D

Sun exposure is a critical factor in vitamin D synthesis. The skin produces vitamin D when exposed to UVB rays. Adequate sun exposure can increase blood levels of vitamin D, which can have positive effects on the immune system. Not being exposed to enough sunlight, especially in winter months and in individuals working in closed environments, can lead to vitamin D deficiency. This may increase susceptibility to viral infections such as respiratory infections. It has been shown in many studies that sun exposure can reduce the risk of infection by increasing vitamin D levels. In the study of Holick[70], it was stated that exposure to sunlight reduces the risk of infection by increasing vitamin D levels.

PHYSICAL ACTIVITY

Physical activity is an important lifestyle factor for overall health and the immune system. Regular exercise can reduce inflammation, increase circulation of immune cells, and improve overall immune function. Exercise can also positively affect the metabolism of vitamin D, which is essential for muscle and bone health. Higher vitamin D levels and a stronger immune system have been observed in individuals who exercise. One study found that individuals who exercise regularly are more resistant to infections[113].

NUTRITION AND VITAMIN D

Apart from magnesium, other nutrients such as calcium, zinc and omega-3 fatty acids are also important for vitamin D activation and immune system functions. Zinc plays a critical role, particularly in antiviral immune responses, and may improve immune functions when used with vitamin D. Omega-3 fatty acids help regulate the immune system by reducing inflammation. Takeda *et al*'s study showed that the combination of zinc and vitamin D improved immune functions and reduced the risk of infection[114].

Vitamin D is a vitamin that is vital for immune system functions. However, it works effectively in conjunction with other nutrients such as magnesium and lifestyle factors such as sun exposure and physical activity. Having these factors together at optimal levels makes the immune system more resistant to viral infections. Vitamin D deficiency, combined with inadequacy of these nutrients and lifestyle factors, can increase vulnerability to infections. Therefore, it is important to take a holistic approach to ensure vitamin D levels are adequate.

CONCLUSION

We investigated the critical function of vitamin D in immune function, particularly in the context of viral infections such as SARS-CoV-2 and influenza. Once associated primarily with calcium and bone health, vitamin D is now known as a versatile steroid hormone with important effects on the immune system. We discuss vitamin D metabolism and the global concern regarding its deficiency and highlight the links between low vitamin D levels and amplified risk of respiratory infections. We also discussed several studies examining the connection between vitamin D levels and the severity and fatality of COVID-19, offering insights into the potential of vitamin D as a prophylactic and therapeutic agent. We think that meticulously investigating the mechanisms by which vitamin D can decrease viral infections, modulation of the RAS, and interactions with respiratory epithelial cells, sheds light on its versatile immune-boosting properties. We also examined the status of vitamin D in fighting influenza. In general, we wanted to provide a comprehensive overview of the important impact of vitamin D on the immune system and its potential effects in the context of viral infections.

FOOTNOTES

Author contributions: Engin MMN and Özdemir Ö have done everything.

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REVIEW

Viral etiologies of acute liver failure

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Abstract

Acute liver failure (ALF) is a rare cause of liver-related mortality worldwide, with an estimated annual global incidence of more than one million cases. While drug-induced liver injury, including acetaminophen toxicity, is the leading cause of ALF in the Western world, viral infections remain a significant cause of ALF and the most common cause in many developing nations. Given the high mortality rates associated with ALF, healthcare providers should be aware of the broad range of viral infections that have been implicated to enable early diagnosis, rapid treatment initiation when possible, and optimal management, which may include liver transplantation. This review aims to provide a summary of viral causes of ALF, diagnostic approaches, treatment options, and expected outcomes.

Key Words: Acute liver failure; Viral hepatitis; Hepatitis B; Hepatitis C; Hepatitis A; Liver disease; Hepatology

Core Tip: Acute liver failure (ALF) is a rare cause of liver-related mortality worldwide, with viral infections remaining a leading global cause. Healthcare providers should be aware of the broad range of viral etiologies that have been implicated to cause ALF given its heterogeneous presentations and high mortality rate. This review aims to provide a summary of known viral etiologies for ALF so that early diagnosis, rapid treatment initiation when possible, and optimal management including liver transplantation can be pursued. Further, this review intends to underscore the importance of further study and characterization of ALF to improve our care and understanding of this condition.

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INTRODUCTION

Acute liver failure (ALF) is a rare condition which presents heterogeneously. ALF is clinically characterized by severe acute liver injury (ALI) for less than 26 weeks in duration, impaired hepatic synthetic function as indicated by an elevated international normalized ratio of greater than 1.5, and encephalopathy in a patient without cirrhosis or other pre-existing liver disease[1]. The development of ALF is associated with high morbidity and mortality and is an indication for emergency liver transplant (LT) evaluation. The global incidence of ALF is estimated to be more one million cases annually[2]. Mortality rates are drastically higher in low and middle-income countries compared to upper-middle and high-income countries[3]. Etiologies of ALF vary considerably between geographic regions. While acetaminophen and other drug-induced liver injury comprise most ALF cases in countries such as the United States and the United Kingdom, acute viral hepatitis remains the most common cause of ALF in many other parts of the world[4]. The precise global burden of viral-induced ALF has historically been difficult to determine given challenges employing diagnostic criteria, accurate diagnosis of viral-infections, and the relative rarity of ALF[3]. Numerous viral pathogens have been implicated in ALF, including hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), hepatitis E virus (HEV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), varicella zoster virus (VZV), parvovirus B19, herpes simplex virus (HSV), yellow fever (YF), dengue virus (DENV), and human adenovirus (HAdV). Table 1 summarizes high-yield points from this review.

HAV

HAV is a nonenveloped RNA virus transmitted by fecal-oral contamination that is endemic in all parts of the world[5]. HAV has an incubation period of about 28 days and typically causes a self-limited diarrheal illness. The acute HAV clinical course consists of a pre-icteric phase of about one week of fever, malaise, anorexia, nausea, vomiting, abdominal pain, and headache followed by an icteric phase which can persist for a month or more[6]. Symptomatic disease seems to be age-related; around 70% of infected adults develop jaundice, compared to 30% of infected children[6]. An estimated 0.1%-0.5% of acute HAV infections progress to ALF[7]. HAV replicates in hepatocytes and the gastrointestinal epithelium. Hepatocellular injury in HAV is attributed to a cytotoxic response to virus-infected cells[8]. Cytotoxic damage from natural killer (NK), NK T-cells, non-HAV specific CD8+ T-cells, and virus-specific cytotoxic T-cells have been shown to contribute to hepatocyte injury and death. There is also emerging evidence that HAV can induce intrinsic apoptosis of hepatocytes in animal models. Virus-specific cytotoxic T-lymphocytes can cause direct hepatocellular injury *via* cell lysis [9]. Non-virus specific lymphocytes are also thought to contribute to hepatocellular injury; this has been demonstrated in a study from Kim *et al*[10] where high levels of interleukin (IL)-15 and upregulation of NKG2D ligands were observed in HAV-infected and uninfected hepatocytes, with severity of liver injury correlating with the activation of non-virus specific CD8+ T cells. Further, studies in mice models have suggested that type I interferon (IFN) receptor-mediated signaling may be involved in intrinsic apoptosis of HAV-infected hepatocytes[9]. HAV-induced ALF can cause the histologic features of massive hepatocyte necrosis, inflammatory cellular infiltrate, and bile ductular proliferation[11]. The precise mechanism underlying progression of severe HAV infection to ALF requires further study.

Diagnosis of acute HAV is made *via* detection of anti-HAV immunoglobulin M (IgM) *via* blood testing in the appropriate clinical context. Anti-HAV IgM can typically be detected 5-10 days before symptom onset. Treatment of severe HAV is supportive, and LT evaluation is indicated for those who progress to ALF[12]. While LT for HAV is extremely rare, reported 5-year post-LT survival in this population is reported to be 69%[4].

Vaccination against HAV has considerably decreased the prevalence of acute infection. The World Health Organization (WHO) recommends universal childhood HAV vaccination in endemic countries and targeted vaccination efforts for high-risk grounds in areas of low endemicity[13]. The combined point-prevalence of HAV-induced ALF in countries with routine HAV vaccination is around 2% (95% confidence interval: 1-3), compared to roughly 27% (95% confidence interval: 13-43) in countries without routine immunization[3]. One study in Argentina showed that the incidence of HAV-induced ALF in children decreased from 54.6% (March 1993 to July 2005) to 27.7% after the implementation of routine single-dose HAV vaccine in 2005 (August 2005 to December 2008). The same study reported zero cases of HAV-induced ALF beyond November 2006[14].

HBV

HBV is a partially double-stranded circular DNA virus and one of the leading causes of chronic hepatitis, cirrhosis, and hepatocellular carcinoma worldwide. It is estimated that < 0.5% to 1% of persons who experience acute or reactivation of HBV will progress to HBV-induced ALF[15,16]. Acute HBV has an incubation period of 1-4 months. Initial illness symptoms can include a serum sickness-like illness followed by anorexia, jaundice, abdominal pain, and constitutional

Table 1 Known viral etiologies of acute liver failure with associated viral genetic material, common symptoms, diagnostic approach, possible treatment options, indication for liver transplant and whether a vaccine is available

Virus	Virus genetic material	Symptoms	Diagnosis	Possible treatment options	Indication for LT evaluation in ALF?	Dedicated vaccine available?
HAV	RNA	Diarrhea, fever, malaise, anorexia, nausea, vomiting, abdominal pain, headache	Serologic	Supportive	Yes	Yes
HBV	DNA	Anorexia, jaundice, abdominal pain, constitutional symptoms	Serologic + viral DNA	Entecavir, tenofovir	Yes	Yes
HCV	RNA	Fatigue, myalgia, low-grade fever, nausea, vomiting, jaundice	Serologic + viral RNA	DAA requires further study in ALF	Yes	No
HDV	RNA	Fatigue, anorexia, lethargy, nausea	Serologic + viral RNA	Requires further study in ALF, demonstrated effectiveness of bulevirtide + peginterferon alfa-2α in chronic HDV	Yes	No
HEV	RNA	Fatigue, anorexia, lethargy, nausea	Viral RNA	Ribavrin, glycyrrhizin	Yes	Yes[2]
CMV	DNA	Pharyngitis, lymphadenopathy, arthralgia, lymphocytosis, splenomegaly, hepatitis	Serologic + viral DNA	Transplantation in ALF; ganciclovir, valganciclovir, foscarnet and cidofovir in ALI	Yes	No
EBV	DNA	Fever, sore throat, lymphadenopathy, mononucleosis syndrome	Viral DNA	Acyclovir, ganciclovir, famcilovir, valganciclovir +/- corticosteroids	Yes	No
VZV	DNA	Blistering, erythematous rash, "shingles"	Viral DNA	Acyclovir, IVIG[1]	Yes	Yes
Parvovirus B19	DNA	Erythema infectiosum: Fever, rash, arthralgias	Serologic	IVIG, hydroxyurea, cidofovir/brincidofovir, coumarin	Yes	No
HSV	DNA	Painful oral or genital sore; non-specific systemic symptoms	Serologic + viral DNA	Acyclovir	Yes	No
YF	RNA	Fever, headache, myalgias, nausea	Serologic + viral RNA	Sofosbuvir[1]	Yes	Yes
SARS-CoV-2	RNA	Upper and/or lower respiratory tract infection, cough, fever, anorexia	Serologic or viral RNA	Supportive	Yes	Yes
DENV	RNA	Fever, headache, body pains, chills, sore throat, rash	Serologic or viral RNA	NAC[1]	Yes	Yes
HAdV	DNA	Localized upper or lower respiratory tract infection, gastroenteritis	Histologic[4]	Cidofovir	Yes	Yes[3]

[1]Requires further study.
[2]Only available in China.
[3]Only routinely available for military recruits.
[4]May require further testing for human adenovirus speciation.
ALT: Acute liver failure; LT: Liver transplant; HAV: Hepatitis A virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HDV: Hepatitis D virus; HEV: Hepatitis E virus; CMV: Cytomegalovirus; EBV: Epstein-Barr virus; VZV: Varicella zoster virus; HSV: Herpes simplex virus; YF: Yellow fever; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; DENV: Dengue virus; HAdV: Human adenovirus; ALI: Acute liver injury; DAA: Direct-acting antivirals; IVIG: Intravenous immune globulin; NAC: N-acetylcysteine; ALF: Acute liver failure.

symptoms[16]. Mortality resulting from HBV-induced ALF is high, with LT-free survival of about 25%[4]. While the pathophysiology of hepatic necrosis is not fully understood, HBV core promoter mutations encourage enhanced viral replication, strong humoral immunity response (evidenced by massive accumulation of IgG and IgM secreting plasma cells in necrotic hepatic tissues of patients with HBV-induced ALF), and intrinsic viral induction of apoptosis may contribute to the development of ALF[12]. Mice models have shown that hepatocellular damage is also mediated by cytokines including tumor necrosis factor (TNF)-α and IFN-γ, sensitizing hepatocytes to immune-mediated damage and exacerbating liver injury[17]. HBV can also have direct cytopathic effects, which has been demonstrated in hepatoma

models expressing HBV large-surface proteins causing vacuole formation and apoptosis and in mice models where HBV core protein has been shown to interfere with hepatocyte mitochondrial recycling, increasing apoptotic hepatocyte death [18,19]. Histologically, HBV-induced ALF is characterized by high production of IgG and IgM secondary to an overwhelming B-cell response with complement deposition leading to massive or sub-massive hepatocyte necrosis[20]. HBV is estimated to cause up to 18% of ALF cases in Europe, 15% in Bangladesh and India, 22% in Sudan, and 7% in the United States[21]. Of note, HBV can cause ALF during an acute infection as well as an acute-on-chronic flare of disease [22].

Increasing use of immunomodulating and immunosuppressive therapies in autoimmune diseases and malignancies have caused significantly more episodes of HBV reactivation, which can occur indirectly through down-regulation of cytotoxic T-cells or B-cell inhibition. Glucocorticoids and anti-TNF therapies are also capable of directly stimulating upregulation of HBV expression[23]. The Association for the Study of Liver Diseases defines HBV reactivation in people receiving cytotoxic or immunosuppressive therapy as ≥ 2 Log increase compared to baseline HBV DNA levels, ≥ 3 Log increase in a patient with previously undetectable HBV DNA levels, new appearance of HBV DNA levels in patients who are HBV surface antigen (HBsAg)-positive and anti-HBV core antigen (anti-HBc) positive, or newly detectable HBV DNA or reappearance of HBsAg in patients who are HBsAg-negative and anti-HBc-positive[24]. Patients who are HBsAg-positive are at higher risk for HBV reactivation than HBsAg-negative and anti-HBc-positive patients. HBsAg-positive patients receiving immunosuppressive/cytotoxic therapy or HBsAg-negative/anti-HBc-positive patients undergoing high-risk therapy (anti-CD20 therapy, stem cell transplantation) are recommended to receive HBV-directed antiviral prophylaxis given the high risk of viral reactivation[24]. In immunocompetent patients with HBV, interruption of HBV-directed antiviral therapy has been associated with a statistically significant risk of HBV-reactivation leading to hepatic failure and increased 28- and 90-day mortality[25].

Diagnosis of HBV as a cause of ALF is largely serologic based on the detection of anti-HBc IgM and HBsAg. HBV DNA polymerase chain reaction (PCR) testing may not result quickly enough to be of clinical utility. The hallmark of diagnosing acute HBV is the detection of anti-HBc IgM; anti-HBc IgM can become detectable 1-2 weeks after the appearance of HBsAg[26]. However, it is important to note that anti-HBc IgM can also reappear during severe reactivation flares of chronic HBV in patients receiving chemotherapeutic or immunosuppressive medications[27]. HBV DNA will be detectable in HBV-induced ALF. Previous studies have found that viral loads are lower at initial presentation for patients who progressed to ALF, perhaps reflecting a more robust immune response predisposing patients to increased hepatocyte damage. A superinfection or coinfection of another hepatitis virus, such as HDV, should also be tested for in those with suspected HBV reactivations[21]. In HBV-induced ALF, antiviral treatment is recommended with entecavir, tenofovir disoproxil fumarate or tenofovir alafenamide[24]. Recent data suggest that early treatment may improve LT-free survival in HBV-induced ALF[28]. HBV-directed antiviral treatment should be continued indefinitely in those who undergo LT or until HBsAg clearance is confirmed[22].

The HBV vaccine is effective in preventing HBV infection and is therefore recommended by the WHO to be included in all national immunization programs[29]. HBV immunization significantly reduces the risk of complications related to HBV, including chronic liver disease, hepatocellular carcinoma, and ALF[30]. In those chronically infected with HBV, achieving functional cure (sustained loss of HBsAg and undetectable HBV DNA after a finite treatment course) has the potential to decrease HBV transmission and thereby the risk of HBV-induced ALF. A primary challenge to HBV functional cure remains the virus' long half-life and covalently closed circular DNA, persistent production of HBsAg from integrated HBV DNA, and host immune exhaustion. The latest approaches to HBV functional cure involve combination therapy aimed to suppress HBV DNA replication and HBsAg production with subsequent stimulation of the HBV-specific immune response. Therapeutic approaches to functional HBV cure currently in testing involve viral suppression with nucleotide or nucleoside therapy in combination with pegylated IFN or immunomodulators[31].

HCV

HCV is a single-stranded RNA virus with seven identified genotypes and 67 subtypes[32]. Acute HCV leads to chronic infection in 70%-80% of cases, placing affected patients at risk of developing cirrhosis and/or hepatocellular carcinoma [33]. Acute infection with HCV is most often asymptomatic but can also present with fatigue, myalgia, low-grade fever, nausea, vomiting and jaundice[34]. HCV is thought to damage hepatocytes through several mechanisms including direct viral injury, secondary oxidative damage and host immune response. HCV can cause direct stress within the endoplasmic reticulum of hepatocytes, with HCV-encoded proteins activating pro-inflammatory molecules such as transcription factor nuclear factor-κB. HCV-infected hepatocytes increase the production of pro-fibrotic signals such as transforming growth factor-. HCV core and NS3 proteins are also thought to promote inflammation by stimulating IL-1 receptor-associated kinase activity, increasing p38 phosphorylation and activating extracellular regulated kinase and c-Jun N-terminal kinase [35]. Further, HCV infection has been shown to induce oxidative stress and cellular damage *via* multiple mechanisms, including of increased production of reactive oxygen species and decreased glutathione stores[36]. HCV also causes hepatocellular injury *via* host immune response, with cytotoxic lymphocytes destroying HCV-infected cells with the release of Fas-ligand and inflammatory cytokines, including IFN-γ and TNF, causing injury to uninfected cells[37].

HCV as an isolated cause of ALF is controversial but has been reported, and acute HCV infection is known to be capable of causing severe ALI, particularly in immunosuppressed patients[12,38]. Severely immunocompromised individuals, such as kidney or LT recipients, are also predisposed to direct HCV-induced viral injury *via* fibrosing cholestatic hepatitis (FCH) which is a rare, rapidly progressive form of cholestatic liver injury with marked jaundice and high HCV viral load[39]. FCH manifests histologically with hepatocyte swelling, cholestasis, periportal peritrabecular

fibrosis, and mild inflammation and can progress to ALF[40]. There has also been documentation of HCV-induced ALF in patients with concurrent chronic HBV infection[41].

The gold standard for diagnosis of HCV infection is detection of HCV RNA by PCR, and routine screening for HCV infection can be performed *via* detection of anti-HCV antibodies. While curative HCV therapy is now widely available, the development of an HCV-vaccine is an active area of research with several neutralizing antibody candidates identified and several vaccine studies ongoing[42]. More research is required to clarify the role of direct-acting antivirals (DAA) in HCV-induced ALF. DAA treatment has shown clinical benefit and sustained virologic response in severe HCV infection including FCH[43].

HDV

HDV is a satellite RNA virus which relies on HBV for viral replication as it utilizes the HBsAg viral envelope for hepatocyte receptor viral entry. A recent systematic review estimated that the global anti-HDV prevalence to be 4.5% in those known to be hepatitis B surface-antigen positive[44]. This translates to a worldwide prevalence of HDV/HBV coinfection of 20-40 million people, although some estimates are as high as 72 millions[45]. Because HDV requires HBV to propagate, infections occur simultaneously as a coinfection or sequentially as a superinfection in patients with a preexisting HBV-infection[44]. Acute HDV infection presents non-specifically following a 3-7 week incubation period with fatigue, anorexia, lethargy and nausea followed by an icteric phase with the appearance of frank jaundice, dark urine and light-colored stools[46]. Mouse models have demonstrated that cytokine inflammation from TNF-α mediates hepatocellular injury in HDV infection[47]. In human cell models, it has also been shown that small HDV antigen may be capable of direct cellular toxicity by binding to mRNA downregulating protective proteins such as glutathione S-transferase P1[48]. Histologically, HDV-induced liver injury is similar to other viral hepatitis infections with hepatocyte necrosis, inflammatory infiltrates with lymphocytes and macrophages, and cytoplasmic eosinophilia[49]. An HDV superinfection in an HBV-infected individual tends to be more severe with a higher chance of progressing to ALF than HBV mono-infection or HBV/HDV coinfection[46].

Diagnosis of HDV is by detection of HBsAg and anti-HDV antibodies. Acute HDV infection is confirmed by the presence of HDV Ag and anti-HDV IgM[26]. However, HDV Ag is not always detectable and cannot necessarily help distinguish between resolved, chronic or acute infections, and thus HDV RNA detection *via* PCR is the gold standard for HDV diagnosis when available[50]. Anti-HBV core IgM is only present in HDV/HBV coinfection and not acute HDV superinfection, providing an ability to distinguish between these two clinical scenarios[45].

As HDV requires HBV to replicate, vaccinations programs for HBV are the most effective measure to prevent HDV infection. There is no HDV-specific vaccine available. In the United States, there are no specific antiviral treatments approved for acute HDV[51]. Until the approval of bulevirtide in Europe in 2020, pegylated IFN-α was the primary drug of choice for HDV infection[45]. Bulevirtide acts by blocking the entry receptor for HBV/HDV on hepatocytes, sodium taurocholate co-transporting polypeptide[52]. A recently published study from Asselah *et al*[53] in patients chronically infected with HDV showed that combination bulevirtide plus peginterferon alfa-2a was superior to bulevirtide alone in achieving undetectable serum HDV RNA. Treatment of HDV should also focus on treatment of concurrent HBV infection [54]. For HDV-induced ALF, data are lacking regarding treatments including bulevirtide, and the definitive therapy is LT [45].

HEV

HEV is a nonenveloped single-stranded RNA virus with eight genotypes that is most often transmitted *via* the fecal-oral route involving contaminated water[55]. HEV is the most common cause of acute viral hepatitis worldwide, with HEV1 and HEV2 (likely human reservoir) usually leading to self-limiting acute viral hepatitis while and HEV3 and HEV4 (thought to be zoonotic viruses with the primary animal host being pigs) have been found to cause chronic hepatitis in immunocompromised patients[55,56]. The estimated incidence of HEV is observed to vary by region, with an estimated 2 million cases annually in Europe and 3.4 million symptomatic cases annually in Asia[56,57]. Hepatitis E outbreaks are usually related to contaminated drinking water reservoirs. Pregnant women are most likely to be affected during outbreaks. Further, acute HEV has been found to progress to ALF more often in pregnant women (22%) compared to non-pregnant women (0%) and men (2%)[58]. Liver injury in HEV-induced ALF is thought to primarily be driven by immune response to HEV infection[59]. HEV is thought to cause hepatocyte damage *via* host immune response in the presence of pro-inflammatory cytokines TNF-α and IFN-γ; direct HEV cytotoxic effects are an active area of inquiry[58]. HEV-induced ALF has been noted to be histologically heterogenous, attributed to the diversity of prevalent genotypes. However, a study of 11 biopsies from HEV-induced ALF showed varying degrees of necrosis, ballooning hepatocyte degeneration (70%), councilman bodies (90%), pseudo-rosettes (70%), Kupffer cell prominence (100%), intracytoplasmic (80%) and canicular (90%) cholestasis, biliary ductular proliferation (90%) and less plasma cell portal inflammation (20%) [60]. While HEV-related mortality is typically comparable to that seen with other acute viral hepatitis infections (0.2%-1%), the mortality rate among pregnant women is estimated to be as high as 20%. The hypothesized mechanism for the increased risk of ALF in pregnancy is related to immunologic changes discouraging antigenic sensitization to the fetus, causing decreases in cellular immunity which predispose to more severe HEV disease[61].

The rapid diagnosis of HEV with indirect immunoglobulin assays is complicated by virologic heterogeneity and lack of understanding of how different HEV genotypes influence the production of diagnostic antigens, making these results difficult to interpret in acute disease[62]. In a recent case series of ALF in the United States attributed to drug-induced liver injury, 9 of 318 cases reviewed had serologic evidence of ongoing acute HEV infection with positive anti-HEV IgM, while 4 of the cases had detectable HEV3 RNA in the serum. Therefore, acute HEV may comprise a small but significant percent of ALF cases of indeterminate cause[63]. Direct testing by HEV PCR, if available, may provide the highly sensitive and specific diagnostic screening test that is required in severe, acute HEV infection[62].

A vaccine against HEV, HEV 239, has been available in China since 2012, but is not presently available in other countries. A phase 3 double-blind placebo-controlled study of this vaccine demonstrated a 10-year efficacy of 83.1% in intention-to-treat analysis and 86.6% in the per protocol analysis and was shown to induce durable anti-HEV antibodies for at least 8.5 years[64]. Despite promising data for HEV 239 in China, the WHO's current position is that available data are insufficient for children under 16 years old as well as cross-protection against HEV genotypes 1, 2 and 3. The WHO does not currently recommend that HEV 239 be incorporated into routine vaccination programs[65]. While there is no established treatment for HEV-induced ALF, ribavirin monotherapy has been shown to encourage viral clearance in immunocompetent and immunosuppressed patients experiencing severe, acute HEV infection. While ribavirin is otherwise contraindicated during pregnancy due to teratogenic effects, case studies have reported normal pregnancy outcomes after fetal ribavirin exposure, suggesting that treatment with ribavirin may outweigh the risk of untreated HEV in pregnancy[66]. There is also low-quality evidence in acute moderate to severe HEV that glycyrrhizin, an extracted component of licorice root, can lead to clinical improvement and normalization of ALT and AST within 30 days of commencing therapy[67]. In chronically infected immunosuppressed solid-organ patients, ribavirin improves viral clearance and sustained virologic response[68]. Unfortunately, a safe ribavirin doses has not been established for HEV-related ALF. Further research should focus on whether the availability of the HEV 239 reduces the incidence of HEV-induced ALF and other associated complications.

CMV

CMV is a double-stranded DNA herpesvirus with a worldwide seroprevalence of 60%-100%. While CMV can cause clinically significant disease with high morbidity and mortality in immunocompromised populations, only 10% of immunocompetent individuals present with symptomatic infection. Reported symptoms are often mild and self-limited, including pharyngitis, lymphadenopathy, arthralgia, lymphocytosis, splenomegaly, and hepatitis[69]. CMV hepatitis causes non-specific symptoms, and cases of ALF attributed to CMV are exceedingly rare[70,71]. While CMV can cause direct cytopathic damage to hepatocytes and cholangiocytes, immune-mediated CD8+ T-cell response and cytokines IFN-γ and TNF has been shown in mouse models to be important to the progression of CMV hepatitis in severe cases[72]. While no diagnostic criteria exist for CMV-induced ALF, an active CMV infection is diagnosed by a detectable CMV DNA PCR test in combination with a positive CMV IgM or an increase in CMV IgG greater than 4-fold the upper limit of normal[69]. Liver biopsy can assist in cases of diagnostic uncertainty. Histologic features of CMV hepatitis include the presence of cytoplasmic and intranuclear inclusion bodies, lobular hepatitis, hepatocellular necrosis, portal mononuclear infiltrates and micro-abscesses[73].

Immunosuppressed individuals, especially solid-organ transplant recipients, are at highest risk of CMV-related complications. There is no CMV vaccine available, and prevention in immunosuppressed individuals is primarily achieved with prophylactic antiviral medications. Valganciclovir and ganciclovir are common choices for CMV viral prophylaxis in LT, and immunosuppressed, patients[74]. While letermovir has been shown to be effective in hematopoietic stem cell transplants, a recently published case series suggests that letermovir may be effective for secondary CMV-prophylaxis in solid organ transplant patients, including LT patients[75]. For CMV-related hepatitis, treatment targets the CMV DNA polymerase and ganciclovir, valganciclovir, foscarnet and cidofovir have been used[73]. However, once CMV-induced hepatic injury has advanced to ALF, it is thought to be unresponsive to antivirals and is therefore an indication for emergent LT evaluation. Suppressive antiviral therapy is essential in patients who undergo LT, as CMV-induced ALF is an identified risk factor for clinically significant CMV disease following LT[69,76]. Further study of comparative effectiveness of treatments for acute CMV-induced hepatitis and whether there are differences in progression to ALF are needed.

EBV

EBV is a double-stranded DNA herpesvirus with a seroprevalence of over 90% worldwide. Infection most commonly causes mild, self-limited fever, sore throat, lymphadenopathy, and mononucleosis syndrome[77]. EBV preferentially infects B lymphocytes by binding the gp350 glycoprotein on CD21. Although EBV can cause a mild subclinical and self-limiting hepatitis as a part of the mononucleosis syndrome, it can also cause ALF. Patients at risk present with a triad of atypical lymphocytosis, splenomegaly, and jaundice. Interestingly, EBV-induced ALI tends to predominantly present in a cholestatic pattern of injury[78]. EBV is nonhepatotropic, and instead is understood to cause hepatic damage *via* EBV-infected lymphocytes with subsequent predominant CD8+ T-cell immune response causing inflammation and hepatocellular damage[79]. Histopathology typically shows diffuse lymphocytic sinusoidal infiltrates in a string of beads pattern, a lymphocytic infiltrate causing expansion of portal tracts, and, in severe cases, massive hepatic necrosis[73]. Immunosuppression is thought to be a risk-factor for severe disease related to viral reactivation in EBV-exposed

individuals[80]. A study from the United States Acute Liver Failure Study Group found that only 4 patients in their database of 1887 patients had ALF attributed to EBV. Of these 4, all were young, immunocompetent adults (< 30 years old) who had been experiencing symptoms for 2-3 weeks prior to presentation[81].

Diagnosis can be challenging given nonspecific pathologic biopsy findings and difficult-to-interpret EBV serum tests (including serum viral load levels)[81]. In terms of diagnostic testing, EBV DNA PCR is more reliable than EBV serologies given the high degree of cross-reactivity to other herpesviruses (*e.g.*, CMV and HEV). EBV IgG, IgM, and heterophile antibody testing can be utilized for initial screening[73]. Ancillary studies such as EBV-encoded RNA histopathologic testing can be used as an additional diagnostic study when available but does not exclude EBV-hepatitis as a diagnosis [82]. Definitive diagnosis of EBV-associated ALF is made by DNA detection by PCR in the appropriate clinical context.

There is currently no EBV vaccine available. Research for an EBV-vaccine is of interest given the virus' implication in multiple malignancies and auto-immune conditions; however, vaccine development is complicated by the virus' complex life cycle, lack of robust animal models, and uncertainty around optimal antigen target and delivery methods[83]. Some of the latest EBV vaccine candidates utilize poxvirus and adenovirus vectors or peptide vaccines[84]. In terms of treatment, the antiviral agents acyclovir, ganciclovir, famcilovir, and valganciclovir, sometimes in combination with corticosteroids, are utilized for EBV-associated hepatitis and severe EBV-disease[69,81]. The relative efficacy of respective antiviral regimens in EBV-induced ALF has yet to be established. However, the United States Acute Liver Failure Study Group recommends initiation of antiviral treatment in severe EBV hepatitis based on available reports. Ultimately, EBV-induced ALF is an indication for LT evaluation[81].

VZV

VZV, or human herpesvirus 3, is double-stranded DNA virus that typically causes the mild disease known as chickenpox. This virus then becomes quiescent in ganglionic neurons and can reemerge as herpes zoster, or shingles, which classically causes a blistering, erythematous, and painful rash in a dermatomal distribution[85]. Disseminated VZV infection is rare and typically only arises in immunocompromised individuals such as those taking immunosuppressive therapies[86]. ALF in disseminated VZV is extremely rare. A literature review from Fang *et al*[86] found 18 case reports, 12 of which occurred in immunocompromised patients. Of the remaining 6 patients, 3 had recently started oral steroid therapy. This study reported a case fatality rate of 77.8%. Documented initial presentations can be nonspecific, including fever, abdominal pain, nausea, vomiting and rash, or isolated chest pain[86]. VZV has been shown to have direct hepatocellular toxicity with intracellular replication with further liver injury thought to be caused by immune-mediate responses to infection[87]. Histologically, necrosis, micro-abscesses, infiltration of inflammatory cells and eosinophilic nuclear bodies have been described[88]. Diagnosis of disseminated Varicella is made by DNA PCR detection of VZV[89].

There are no guidelines for treatment of VZV-induced ALF. Acyclovir is typically administered when VZV-induced ALF is considered, and delays in its initiation should be avoided. Fang *et al*[86] found that of 3 patients with ALF who received intravenous immune globulin, 2 survived, suggesting a possible benefit. Given the rarity of VZV-induced ALF in immunocompetent patients, the American Gastroenterological Association conditionally recommends against routine VZV screening in immunocompetent patients presenting with ALF[90]. VZV-induced ALF is an indication for LT evaluation, and outcomes have been reported to be favorable[91].

The VZV vaccine is a live-attenuated vaccine which is recommended for all healthy patients. The WHO recommends including the VZV vaccine in all universal routine vaccination schedules[92]. The VZV vaccine is not recommended for highly immunocompromised patients including primary immunodeficiency, solid organ transplant recipients, patients receiving active chemotherapy, human immunodeficiency virus infection with CD4 cell count < 200 × 10^9/L, high dose corticosteroid therapy ≥ 20 mg prednisone for at least 14 days, and some stem cell transplant patients owing to concerns regarding low likelihood of response[93]. While widespread VZV vaccination remains a WHO public health goal, trends in epidemiology and cost-effectiveness of the vaccination have led to slow adoption of universal VZV vaccination[94].

PARVOVIRUS B19

Parvovirus B19 is a single-stranded DNA virus and part of the *Parvoviridae* family that infects and replicates in erythroid precursor cells. It is commonly known to cause erythema infectiosum or "fifth disease", characterized by fever and rash in children as well as rash with clinically significant arthralgias in adults[95]. Parvovirus B19 can also infect hepatocytes and other cells that possess globosides and glycospingolipids in their cell membrane and induce apoptosis[96]. Parvovirus B19 is thought to cause hepatocyte damage primarily through direct cytopathology of the NS1 protein which is capable of binding and cleaving both host and viral and host DNA, resulting in damage that ultimately leads to hepatocyte apoptosis *via* the caspase pathway[97]. Histologically, these mechanisms are reflected in parvovirus B19-induced ALF biopsy specimens which demonstrate confluent necrosis with evidence of widespread hepatocyte apoptosis referred to as hepatocellular dropout[98].

While hepatitis related to parvovirus B19 infection is estimated to occur in 4.1% of patients infected, parvovirus B19-induced ALF remains an extremely rare clinical entity[96]. Diagnosis of acute parvovirus B19 disease relies on detection of anti-parvovirus B19 IgM, which may not become detectable until 10-14 days post-infection. Direct testing for parvovirus B19 DNA may be the preferred method in immunocompromised individuals or those with suspected severe acute infection[26]. Most of the literature relating to ALF in acute parvovirus B19 infection involves children. Case reports in adults have described an acute hepatitis with spontaneous remission, indicating that parvovirus B19 disease is

generally less severe in this population. Treatment of severe parvovirus B19 is typically symptom-directed (*e.g.*, transfusions to treat anemia, non-steroidal anti-inflammatory drugs for arthralgia, *etc*). Studies regarding management of parvovirus B19-related ALF are lacking, although administration of large-doses of human intravenous immune globulin are thought to contain high levels of anti-parvovirus B19 antibodies capable of reducing viral load[99]. Novel therapies actively being studied for parvovirus B19 include hydroxyurea given its antiproliferative effect on erythrocytes, cidofovir and brincidofovir given their broad activity against DNA viruses, and newer coumarin derivatives and flavonoid molecules. However, these candidates are not being studied specifically in parvovirus B19-associated hepatitis or ALF [99]. Future study of these treatments may inform whether they also reduce the incidence or severity of liver injury in parvovirus B19 infection. LT evaluation and transplantation has been reported for parvovirus B19-induced hepatitis progressing to ALF[100,101]. There are no currently available vaccines against parvovirus B19, although development of a vaccine is an area of active inquiry[102].

HSV

HSV types 1 and 2 are double-stranded DNA viruses. Infections with HSV are common, with estimates in 2016 of 491 million people living with HSV type 2 (genital) infection and 3583.5 million living with HSV type 1 (oral) in people younger than 50 years old[103]. HSV-induced hepatitis is a rare complication which commonly leads to ALF in 74% of cases. It is associated with a mortality rate of 90% due to delayed diagnosis and treatment. Diagnosis of HSV hepatitis can be complicated by non-specific presenting symptoms. For example, more than half of patients with HSV-associated hepatitis present without mucocutaneous lesions. Anicteric hepatitis with low or normal bilirubin levels but profound transaminase elevations is common in HSV-induced hepatitis and ALF[104]. HSV has direct cytopathic effects, with mouse models showing that infection results in DNA-fragmentation and activation of caspase-3 enzyme and Fas-ligand, facilitating rapid hepatocyte apoptosis[105]. Histologically, HSV-induced ALF is characterized by coagulative necrosis, Cowdry's type A intranuclear inclusion bodies, evidence of hepatocyte apoptosis and inflammatory cell infiltrates (notably lymphocytes and plasma cells)[106].

Diagnosis of HSV-induced ALF can be challenging. In a small study of patients with ALF (n = 63), 6 of the patients with an indeterminate cause of ALF and 1 of the patients with pregnancy-related ALF were found to have positive HSV IgM antibodies but a negative HSV-PCR, while all 4 of the patients with confirmed HSV-induced ALF had high viral load by HSV PCR (only 2 of which had a positive HSV IgM)[107]. This indicates that when HSV-associated ALF is suspected or the diagnosis is unclear, detection of HSV DNA in the blood by PCR should be pursued early. While no specific guidelines exist for treatment of HSV-induced ALF, acyclovir treatment has been associated with reduced mortality and a decreased need for LT[69]. Further, pregnant patients and neonates are at higher risk for developing disseminated HSV, with mortality rates in pregnant patients approaching 40% in HSV-induced hepatitis. Acyclovir has been shown to have favorable survival outcomes in HSV-induced hepatitis in pregnancy and be of no additional risk to the fetus, justifying early empiric administration in pregnant women when HSV-induced hepatitis is suspected[108]. LT evaluation is indicated for HSV-associated ALF, and lifelong suppressive antiviral therapy is recommended following transplant given the risk of recurrence of disseminated HSV[69].

There is no vaccine available for HSV type 1 or 2. Vaccine development to date has been hampered by viral latency and HSV's ability to evade the innate and adaptive immune system. While a preventative vaccine against HSV is the ultimate goal, there is also active research into therapeutic vaccines that may discourage reactivation of latent infection. There are presently multiple vaccine candidates against HSV in various stages of development[109].

YF VIRUS

VF virus is a mosquito-borne positive-single-stranded RNA flavivirus. YF is endemic to areas of Africa and Central/South America. It is transmitted by the *Aedes* mosquito in Africa and the *Haemagogus* or *Sabethes* species in South America. YF virus presents primarily with mild and self-limited disease characterized by fever, myalgias, headache, and nausea following an incubation period of 3-6 or up to 15 days[110]. YF can directly infect hepatocytes, with a study from da Costa Lopes *et al*[111] demonstrating that hepatocyte injury in VF infection may be associated with increased apoptosis, as evidenced by the presence of associated markers, caspase 3, caspase 8, BAX, Fas, Fasl, granzyme B and survivin. Histologically, YF-induced liver injury shows lytic midzonal hepatocyte necrosis, hepatocyte apoptosis, and portal and acinar inflammatory infiltrate disproportionate to the extent of hepatic injury observed[112].

Diagnosis of YF is typically based on clinical suspicion and requires serologic confirmation *via* detection of VF IgM or YF RNA PCR[113]. Around 12% of patients develop severe YF with involvement of various organ systems, including ALF [110]. Once severe YF develops, the treatment is supportive. IFN-α and high-dose ribavirin have not proven to be effective [114]. There is emerging evidence that the anti-HCV antiviral, sofosbuvir, may have therapeutic benefit in severe YF-related disease. This remains an area of study in Brazil given the ongoing YF outbreak in the country[115,116]. LT for YF-induced ALF has been reported in small studies. One such study of 7 LT patients with YF-induced ALF showed that 3 survived, while the 4 who died had histologic evidence of graft-infection with YF[117]. Further study of patients who undergo LT for YF-induced ALF is needed.

A live virus vaccine is available against YF. The YF vaccine is recommended for those aged ≥ 9 months who are traveling to or living in endemic areas; the vaccine is contraindicated in people with a history of thymoma/thymus dysfunction, acquired immunodeficiency syndrome, or those receiving immunosuppressive drugs or chemotherapies

[118,119]. The WHO recommends routine YF vaccine to all infants ≥ 9 months, at the same time as the measles vaccine in areas with reported YF cases, and for YF surveillance so that reactive mass-vaccination programs can be launched in response to outbreaks[120]. The YF vaccine is intended to reduce the risk of people developing severe YF-disease[119]. Further study to understand whether vaccination reduces the incidence of YF-induced ALF is needed, although the low number of annual events poses a barrier to the timeliness of reaching this conclusion.

SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2

The novel strain of coronavirus, an RNA virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19). While ALF has been observed in patients with COVID-19 disease, treatment with hepatotoxic medications including remdesivir and acetaminophen as well as hypoxic liver injury secondary to pulmonary disease and micro-thrombosis may be responsible for these cases as opposed to a direct hepatotoxicity of SARS-CoV-2 virus[121,122]. A potential mechanism for direct viral injury to hepatocytes involves binding to the angiotensin-converting enzyme 2 receptor on cell surfaces following by S-protein activation by transmembrane protease serine 2, both of which are present on liver parenchymal cells and cholangiocytes. While COVID-19-related hepatitis and cholangiopathy are well documented, case reports of ALF without respiratory disease are very rare. Furthermore, pathologic analyses of related liver specimens have demonstrated confounded by features of hypoxia or ischemia; studies to date imply that direct viral infection of the liver is uncommon in COVID-19[123]. The pathogenicity of SARS-CoV-2 as a potential cause of ALF requires further study.

COVID-19-induced ALF has sparing case reports, with the two identified for this review documenting resolution of ALF without requiring LT[124,125]. However, there are documented cases of COVID-19-associated cholangiopathy and sclerosing cholangitis requiring LT evaluation and ultimate organ transplantation[126,127]. Further study of SARS-CoV-2 is needed to clarify risks and natural course of hepatobiliary manifestations of COVID-19.

Vaccines against SARS-CoV-2 remain widely available in the United States. On 5 May 2023, COVID-19 was no longer considered a public health emergency by the WHO; given high rates of population immunity the organization only recommends routine revaccination in special populations including pregnant people, older adults, those who are immunocompromised, and healthcare workers[128]. Vaccination has been shown to reduce the severity of COVID-19 infection and reduce the risk of liver function abnormalities in patients with metabolic-dysfunction associated steatotic liver disease[129]. Available research does not address whether SARS-CoV-2 vaccination modifies individual risk for developing ALF in COVID-19 disease but may be an area of future inquiry.

DENV

DENV is an RNA virus carried principally by the *Aedes* mosquito vector in tropical and subtropical regions of the world. It is caused by a flavivirus with four distinct subtypes (DENV-1, DENV-2, DENV-3, and DENV-4) defined by different antigens. DENV is estimated to have an incidence of around 400 million cases and causes 22000 deaths worldwide per annum[130]. DENV classically presents in the febrile phase as an acute illness with headache, body pains, chills, sore throat, rash (petechiae or ecchymosis) and laboratory evidence of thrombocytopenia and leukopenia. DENV can defervesce and resolve or enter the critical phase of illness with severe capillary leakage, shock and organ damage[131]. The reported incidence of DENV-induced ALF in adults ranges from 0.31%-0.71%, with younger adults being at higher risk[132]. The pathophysiology of ALF in DENV fever is hypothesized to occur *via* direct cytopathic effects of the virus infecting endothelial cells, Kupffer cells and hepatocytes causing cell damage and apoptosis and indirectly *via* host immune response to infection, microvascular leakage and shock[133].

Diagnosis of dengue is most commonly made by detection of dengue RNA or NS1 antigen during the first 7 days of illness or *via* DENV IgM after day 4[130]. While treatment of DENV is typically supportive, small studies in DENV-induced ALF suggest that N-acetylcysteine (NAC) may be beneficial in this population. Giri *et al*'s scoping review found a retrospective cohort study of 33 pediatric patients with DENV-associated ALF in Thailand that demonstrated a recovery rate of 75% for those treated with NAC *versus* 53% in those who received standard medical treatment, although the results were not statistically significant[132]. Another case series found a potential benefit of NAC in reducing the severity of ALF in DENV infection but no survival benefit[132]. LT for DENV-induced ALF is not common but has been reported. A recently published case series from Rajakumar *et al*[134] reported four cases of DENV-induced ALF, two of which recovered spontaneously and two required LT with one dying immediately post-transplant. Clinicians evaluating a patient for ALF should consider DENV as a cause if a patient has recently traveled to an endemic area.

There is a DENV vaccine available, CYD-tetravalent DENV vaccine, which has been shown to reduce the risk of severe DENV disease in secondary or subsequent infection where the risk for severe disease is higher[135]. The WHO recommends the use of CYD-tetravalent DENV vaccine in individuals ≥ 9 years old with serologic evidence of a previous DENV infection. In May of 2024 with the advent of data from the TAK-003 DENV vaccine, the WHO updated their recommendation encouraging countries to consider the introduction of TAK-003 into routine immunization programs, targeting children aged 6-16 years, in areas of high DENV transmission[136]. Further study is needed to understand if countries adopt TAK-003 in line with the WHO's recommendation, and whether routine administration of vaccines confer protection against DENV-induced ALF.

HADV

HAdV is a non-enveloped double-stranded DNA virus which can cause mild, localized upper or lower respiratory tract infections, keratoconjunctivitis, and gastroenteritis in immunocompetent hosts[137]. HAdV has more than 100 genotypes and 52 serotypes identified to date. HAdV has been further classified into seven species, HAdV-A through -G, with different species demonstrating different affinities for various tissues of the body[138]. HAdV species A, F and G target the gastrointestinal tract and are known to cause gastroenteritis and diarrhea. In 2022, there was a documented outbreak of severe hepatitis in otherwise healthy young children in countries around the world, with more than 1000 children requiring hospitalization. In the United States, the CDC reported 6% of children in this cohort ultimately required LT, and 4% died. 299 of these patients were tested for HAdV, with 45% testing positive. HAdV-F41 was the most common HAdV species identified[139].

HAdV as a cause for severe ALI or ALF is extremely rare, predominantly occurring in immunocompromised hosts such as solid organ transplant recipients, bone marrow transplant recipients, or patients receiving active chemotherapy [140]. HAdV-induced ALF has a high mortality rate, estimated around 60%[137]. While not entirely understood, HAdV causes liver injury *via* direct cellular cytotoxicity and apoptosis, with viral uptake in hepatocytes and Kupffer cells having been demonstrated to be facilitated at least in part by viral binding to coagulation factor IX and complement component C4[141]. Further, HAdV is known to cause liver toxicity *via* immune-mediated mechanisms in response to IFN-γ and C-X-C motif ligand 9 production and signaling[142]. Diagnosis of HAdV-induced ALF can be established *via* liver biopsy, with possible histopathologic findings showing nonzonal coagulative hepatic necrosis, hepatocyte and intranuclear viral inclusions, and possible lack of inflammation[143]. When available, serum HAdV viral load may help support the diagnosis of HAdV-induced ALF in the appropriate clinical context[137].

Routine HAdV vaccination is only recommended for military recruits in the United States, who obtain a live oral vaccination for HAdV type 4 and type 7 to prevent febrile acute respiratory disease[144]. The WHO does not have specific HAdV vaccination recommendations. For treatment of HAdV-induced ALF, there is some indication, primarily in pediatric patients with ALI/ALF, that early administration of cidofovir may be an effective therapy[145,146]. In severe disease that has progressed to ALF, LT evaluation is warranted, and good graft function and post-transplant survival has been reported[147]. HAdV should be included as part of an initial broad diagnostic evaluation in a patient with a clinical and laboratory picture concerning for ALF, particularly in the setting of a viral-like illness. Further research of HAdV, and especially research into species and subspecies known to have gastrointestinal and hepatic tropism, is needed to understand whether HAdV is an underrecognized cause for ALI and ALF.

CONCLUSION

ALF is a rare, highly morbid, and fatal condition. A variety of acute viral infections are capable of causing massive hepatic necrosis which can lead to ALF. When ALF is suspected, a broad diagnostic workup including viral and non-viral causes is necessary for rapid diagnosis, appropriate treatment, and possible referral for LT evaluation. Further research involving novel diagnostic approaches, treatment efficacy, and patient outcomes in ALF will be critical to advancing our understanding of this heterogenous condition.

FOOTNOTES

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MINIREVIEWS

Dengue outbreaks in northern Nigeria: Evaluating the recommended Takeda vaccine and future prevention strategies

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Abstract

Dengue fever, caused by the dengue virus (DENV), poses a significant public health challenge globally, with Nigeria experiencing sporadic outbreaks. A clear understanding of the dengue burden has not been achieved in Nigeria, just as in other African countries. Understanding the epidemiology and burden of dengue fever is essential for effective prevention and control strategies. This paper examines the recent dengue outbreaks in northern Nigeria, particularly in Sokoto state, and evaluates the recommended Takeda dengue vaccine (TDV) along with future prevention strategies. Despite limited surveillance and underreporting, dengue fever is endemic in Nigeria (with over 5 million cases and 5000 dengue-

related deaths in 2023), with recent outbreaks indicating a growing concern. The TDV, a live attenuated tetravalent vaccine, has shown promise in preventing dengue fever, but challenges such as vaccine acceptance and accessibility need to be addressed. Global urbanization contributes to the disease's spread, which is influenced by factors such as population density, cultural beliefs, water storage practices, hygiene, and water supply accessibility. Future prevention strategies must focus on government intervention, community practices, and innovative vector control measures to mitigate the spread of DENV in Nigeria. This study will serve as a valuable reference for policymakers, researchers, and clinicians in the management and control of DENV in Nigeria and Africa as a whole.

Key Words: Dengue outbreaks; Northern Nigeria; Takeda dengue vaccine; Vaccine acceptance; Epidemiology

Core Tip: Dengue fever, a persistent public health concern globally, remains endemic in Nigeria, with recent outbreaks stressing the urgency for effective prevention and control. Understanding the epidemiology and root causes of dengue outbreaks in Nigeria, particularly in Sokoto state, is important for targeted intervention. The recommended Takeda dengue vaccine holds promise in mitigating the disease burden, but challenges such as vaccine acceptance and accessibility persist. Future prevention efforts should prioritize government intervention, community practices, and innovative vector control measures to curb dengue transmission. This study provides insights for policymakers and healthcare professionals in addressing the dengue challenge in Nigeria.

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INTRODUCTION

With over 5 million cases and 5000 dengue-related deaths in 2023, dengue remains a significant public health challenge worldwide. The transmission of dengue viral infection (DVI) from mosquitoes to humans is particularly prevalent in tropical and sub-tropical climates, with the rise in cases linked to global warming[1]. Nigeria, experiencing hyperendemicity from 2009 to 2020, now faces an endemic status across almost all states, with Sokoto state reporting the latest outbreak in the Northwestern region[2-4]. Global urbanization contributes to the disease's spread, which is influenced by factors such as population density, cultural beliefs, water storage practices, hygiene, and water supply accessibility.

The recently recommended Takeda's dengue vaccine by World Health Organization (WHO) experts offers promise in disease eradication. Government initiatives, coupled with improved social and environmental conditions, could alleviate the burden of the disease, particularly in Nigeria. Public awareness campaigns focusing on personal and environmental hygiene, alongside education about the impact of climate change, are crucial steps toward mitigating dengue's impact[5, 6]. This paper conducts a comprehensive analysis of the recurrent DVI outbreaks in northern Nigeria, investigating their root causes and presenting updated mortality and natality rates. Additionally, this study proposes innovative solutions to address the frequent outbreaks, emphasising future prevention strategies. A critical evaluation of the recommended Takeda Vaccine is included, highlighting its significant impact in combating the disease within the Nigerian context.

OUTBREAKS OF DENGUE IN NORTHERN NIGERIA AND DISEASE ESTIMATES

Despite the reported occurrence of dengue outbreaks globally, and Africa being among the top leading regions, in Nigeria the disease outbreak has been sporadic[7,8]. Although the initial cases of dengue have been dated to the 1960s[9], there are still limited available surveillance reports on the dengue cases in Nigeria. Based on the studies of Emeribe *et al* [6], from 2009 to 2020, 30 (3.9%) cases of dengue were attributed to the south-south, 74 (77.1%) to south-east, 534 (37.6%) to north-west, 402 (34.3%) to south-west, 413 (23.5%) to north-central, and 93 (9.2%) to north-east (the least in the country) parts of the country[10]. The most recent dengue fever outbreak in Nigeria was reported in November 2023 in Sokoto state with a total of 13 confirmed cases and 71 suspected cases. Neither case of severe dengue nor death was documented [11]. Moreover, a clear understanding of the dengue burden has not been achieved in Nigeria, just as in other African countries[8]. This is due to the similarity of symptoms with other tropical diseases (such as malaria), insufficient laboratory detection, confirmation capacity, and shortfalls in surveillance and case reporting[12,13]. Generally, dengue fever has been reported to be endemic in Nigeria[10,14].

In Nigeria, there is inadequate surveillance for dengue because it is not very well understood by the medical community, as evidenced by the misdiagnosis and underdiagnosis of the viral infection in numerous unclassified febrile illnesses. However, Nigeria's dengue disease burden may be drastically underestimated[4]. A summary of prevalence

study characteristics of dengue virus (DENV) infection in Nigeria showed the highest prevalence significantly in the south-eastern (77.1%), north-west (34.3%), and north-central (23%) parts of the country, while the north-east and south-south parts had the least at 9.2% and 3.9%, respectively[2,15]. This shows a higher prevalent rate of disease in the southern compared to the northern states. Also, it might be concluded that dengue fever is hyper-endemic in Nigeria.

The most recent outbreak occurred in the north-western part of the country, Sokoto. No fewer than 13 cases were confirmed out of 71 suspected cases. However, no death was reported[2]. The multisectoral National Emerging Viral Hemorrhagic Disease Technical Working Group (NEVHD-TWG), which is directed by the Nigeria Center for Disease Control and Prevention (NCDC), has worked with partners and pertinent stakeholders to conduct a quick risk assessment to guide in-country preparedness efforts. The NEVHD-TWG is in charge of organizing preparations for new viral hemorrhagic fever infections, such as the Ebolavirus disease. Using a dynamic risk assessment, it has been established that the dengue outbreak's current risk level is moderate because only one state (Sokoto) has reported confirmed cases and out of the 23 Local government areas of the state, only three were affected with no death. Additionally, the state can draw on the knowledge gained from previous DENV outbreaks (2015-2019) to respond to the outbreak[2,16].

GLOBAL EPIDEMIOLOGY OF DENGUE VIRUS DISEASE

DENV disease, though regarded as a 'neglected tropical disease', has been the most important arboviral disease in the world. The disease is widely distributed around the world, in both tropical and subtropical regions and also in both urban and suburban areas, as more than 50% of the world's population live in regions where this disease can potentially occur. The earliest documented symptoms consistent with dengue appeared in a Chinese medical encyclopedia in 992 AD. However, these records were initially published by the China Dynasty centuries earlier (265–420 AD) before being formally edited. The disease was described as 'water poison' and was linked to flying insects[17]. Inadequate vector control, urbanization, excess international travel, climate change, and unavailability of effective antiviral drugs and vaccines to prevent the disease, are what increased the distribution, endemicity, and epidemicity of the DENV around the world[18].

Although the global estimates of DENV disease differ for the last 50 years, the incidence of the disease has increased 50 times annually, thereby making the number of reported cases increasing from 2.2 million in the year 2010 to 3.2 million in 2015. About 3.9 billion people living in 128 countries of the world are at risk of DENV disease, and reports had it that before 1970, severe DENV disease epidemics had occurred in only nine countries, but as of now, the disease is endemic in more than 100 countries of the world, in almost every continent[16]. It was reported that approximately 400 million cases of DENV disease take place each year around the world, where manifestation of symptoms is seen in 96 million of the cases. The WHO reported that 500000 cases of DENV disease occur every year around the globe, leading to approximately 22000 deaths annually, although only 5%-20% of the mortality rate is reported in some regions. The countries that are most affected are those from the Southeast Asian, Western Pacific, and American regions[5,14].

The first outbreak of DENV disease was identified in the year 1779, which occurred in two prominent capital cities, Cairo in Egypt and Jakarta in Indonesia. Moreover, a year later in 1780, another outbreak was confirmed in North America, which was the Philadelphia outbreak[12]. In North and South America, over 1.6 million cases of DENV disease were reported in 2010, where 49000 of the cases were severe cases. The largest outbreak of DENV disease occurred in the United States in 2016, whereover 2.38 million cases were reported, and during this outbreak, the cases were more prevalent in Brazil, with almost 1.5 million cases[18].

Likewise in Africa, the disease was reported in the Eastern, Western, and Southern parts of the continent, since the beginning of the 19th Century[8]. From 1960 to 2010, reviewed data has shown that 22 countries in Africa have reported random and patternless cases of DENV disease, of which 20 reported confirmed cases in the laboratory and two reported clinical cases only[19]. In Asia, the outbreak of DENV disease started in the southeast part of the continent, after World War II, as a result of urbanization[20]. In 1953 and 1956, two cases of dengue outbreaks respectively occurred in the Philippines, which were the first reported cases in Asia. From 2004 to 2010, a large number of cases were reported in Indonesia, which was the second in number of cases after Brazil. Many researchers have agree that the cases of DENV disease will eventually increase in due time to come, due to the globalization and expansion and increased reports being received from the WHO[16].

BIOLOGY OF DENV AND DISEASE DESCRIPTION AND TRANSMISSION

DENV is an arthropod (mosquito) borne pathogen responsible for the causation of the disease termed dengue. DENV is mainly transmitted by a specific species of mosquito called the *Aedes* mosquito[15,21]. DENV is a flavivirus belonging to the genus *Flavivirus* and the family *Flaviviridae*. The different common existing serotypes of the virus are DENV-1, DENV-2, DENV-3, and DENV-4[1]. Dengue fever typically has a sudden onset of symptoms, including high fever, severe headache, pain behind the eyes, muscle and joint pain, nausea, vomiting, and rash. Infection with one serotype of the DENV confers life-long immunity to that serotype but only temporary immunity to the other serotypes, increasing the risk of severe disease upon subsequent infections with different serotypes[5]. Moreover, antigenically different forms of the virus also exist, having varying structural and non-structural proteins[22]. The DENV is a single-stranded positive (+)-sense RNA virus, with an approximate size of 50 nm diameter and 10700 bases. Other similar arthropod-borne families of the virus include Zika, Japanese encephalitis, tick-borne encephalitis, yellow fever, and West Nile viruses[16, 23].

The *Aedes* species, particularly *Aedes aegypti* and *Aedes albopictus*, are common vectors of DENV[15,21]. DENV is primarily transmitted (Figure 1) through the bite of infected *Aedes* mosquitoes, with humans as the main reservoir and amplifying host. Non-human primates can host the virus but are not the primary mode of human transmission. Transmission of all four DENV serotypes (1, 2, 3, and 4) occurs mainly through human-mosquito-human cycles with mosquitoes being the primary vectors[12].

Human infection of DENV is following the bite by the *Aedes* mosquito that has fed on an already infected person[1]. After the feeding, the virus develops within the mosquito gut (having an incubation period of 10-12 d) and then disseminates to other parts of the mosquito. For the rest of the mosquito's life span, once it has fully become infectious, it can continue to transmit the disease (*via* horizontal transmission)[23,24]. Even though rare, the vertical (maternal) transmission of the virus from pregnant mothers to their babies has been reported. Other rare modes of transmission are through sharp objects (*e.g.* needles), and organ and blood product donations[12]. DENV disease ranges from mild and asymptomatic form to severe shock syndrome and severe haemorrhagic fever. The disease can manifest in varying severity, from mild flu-like symptoms to severe forms such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which can be fatal if not promptly treated. Severe DHF ensues because of a heterologous infection. The severe form of the disease presents a flu-like symptom and a host can be infected multiple times[25]. The most common signs and symptoms are headache, fever, pains (in joints and bones), myalgia, and mucosal bleeding in rare cases[26]. Moreover, life-long immunity to a specific serotype may be conferred[23] coupled with short-term immunity to other related serotypes[5].

UNIQUE ENVIRONMENT AND MOSQUITO VECTOR CHARACTERISTICS IN NORTHERN NIGERIA

Northern Nigeria experiences a tropical climate characterized by high temperatures and seasonal rainfall, creating favourable conditions for the breeding of *Aedes* mosquitoes and the transmission of the DENV. Urban areas in northern Nigeria, such as Kano, Kaduna, and Sokoto, are particularly susceptible to dengue transmission due to factors such as rapid population growth, unplanned urbanization, and inadequate sanitation infrastructure. The presence of densely populated urban slums with poor housing conditions and limited access to basic amenities can facilitate mosquito breeding and increase human-mosquito contact, amplifying the risk of dengue transmission[18,27].

Aedes mosquitoes, especially *Aedes aegypti*, are well-adapted to urban environment and are the primary vectors of DENV transmission in northern Nigeria. They have distinctive physical characteristics, including black and white stripes on their legs and body, and prefer to breed in artificial water containers found in and around human dwellings[10]. Common breeding sites for these mosquitoes in northern Nigeria include discarded water storage containers, flowerpots, and other containers that collect stagnant water. They exhibit daytime biting behavior, with peak activity during the early morning and late afternoon, increasing the likelihood of human-mosquito contact and dengue transmission[2].

CAUSES OF DENGUE VIRAL DISEASE IN NORTHERN NIGERIA

Over 2.5 billion population live in dengue-endemic countries worldwide, and roughly 390 million people have been infected with DENV[28,29]. In those regions, approximately 50-100 million new cases are reported each year[9]. DENV is spread by the primary vector, *Aedes aegypti,* and the less efficient vector, *Aedes albopictus.* Increases in temperatures are causing the vector to spread all over the world, which thereby facilitates the transmission of dengue to previously unreported countries. This has contributed to the prevalence of dengue fever and other arboviral infections[8]. The disease is most prevalent in tropical and subtropical climates, leaving about one-third of the global population vulnerable [30]. Exposure to DENV causes a variety of clinical conditions that ranges from mild asymptomatic dengue fever to severe DHF and DSS, which can be deadly[25].

The significant spread of DENV in northern Nigeria (Sokoto) is a result of a lack of effective mosquito control and prevention measures. Dengue fever has reemerged as one of the world's most common mosquito-borne diseases. Dengue fever is currently endemic in 128 countries, the majority of which are developing countries. A recent dengue distribution model predicted 390 million dengue infections annually, with 96 million cases emerging[29]. Sokoto has recently reported confirmed cases of dengue from 3 Local government areas[2], thus calling for an urgent need to strengthen sero-surveillance so that authorities can effectively prepare for an outbreak.

Humans contract dengue fever from female *Aedes* mosquitoes, a vector belonging to the subgenus *Stegomyia. Ae. aegypti* that has been the primary epidemic carrier in the tropical and subtropical regions while it has been discovered that certain species, including *Aspergillus niveus* (*Ae. Niveus*), *Aedes albopictus* (*Ae. Albopictus*), *Aedes polynesiensis,* and members of the *Ae. scutellaris* complex, exist as secondary vectors[16]. The life cycle of the *Aedes* mosquito takes up to 8 to 10 days at room temperature, depending on how often it feeds. It has two phases: The terrestrial phase (eggs, adults) and the aquatic phase (larvae, pupae). However, *Ae. niveus* is only thought of as a sylvatic vector. *Ae. albopictus* has become an increasingly significant vector due to its ease of adaptation to new habitats, particularly in temperate zones. As a result of its spread to *Ae. aegypti*-free countries, DENV now have more regions to infect and transmit the disease. But even so, its role in human DENV infections remains negligible[30,16].

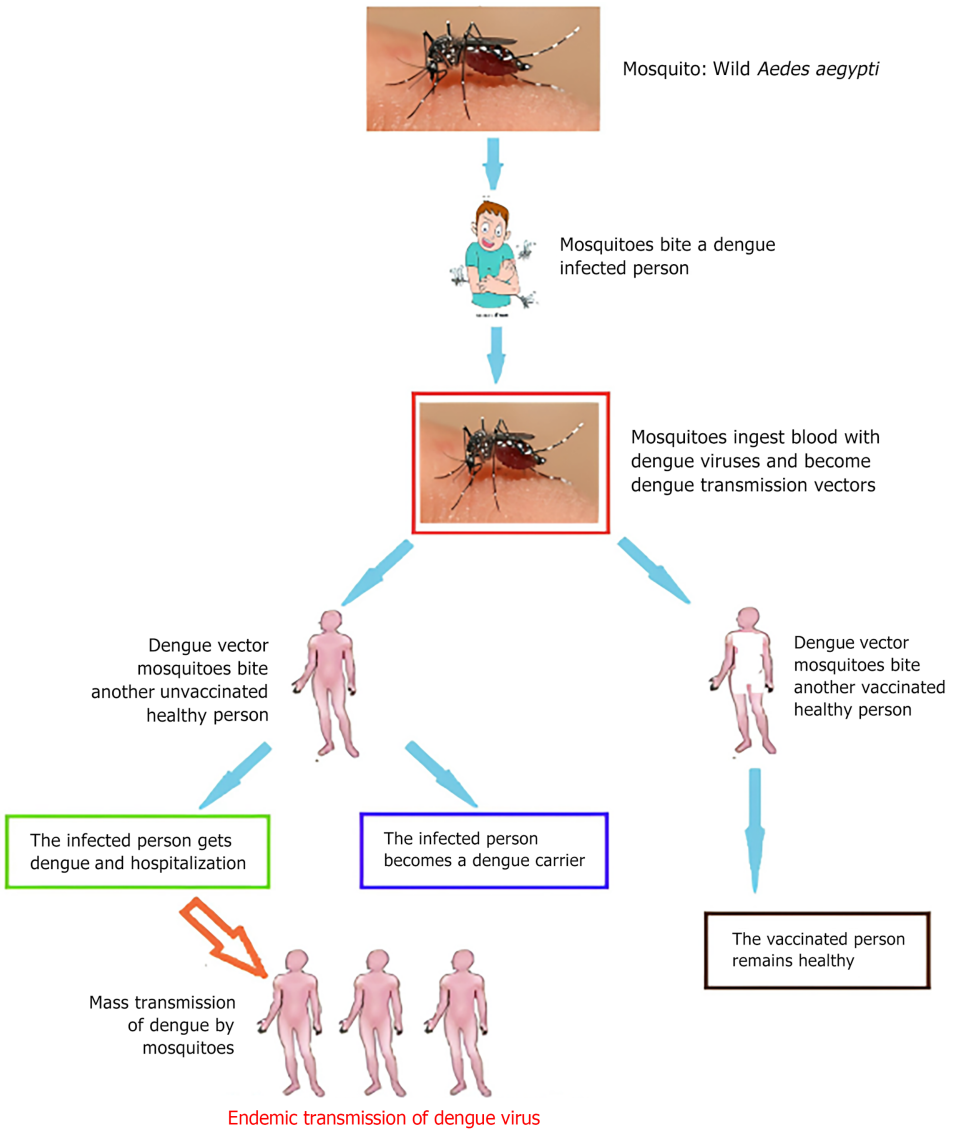

Figure 1 Dengue transmission cycle. The transmission cycle of dengue virus typically begins with a mosquito bite and involves various stages such as mosquito bite, blood meal and viral acquisition, transmission to humans, and symptomatic phase until an infected person requires hospitalisation.

RISKF ACTORS FOR DENV

The associated risk factors for DENV infection include travel to endemic areas, poor sanitation, stagnant water, lack of mosquito control measures, urbanization, previous infection, immunocompromised status, and variations in the climates (most especially) of tropical and subtropical regions[3,12]. Other factors contributing to the spread of dengue have been attributed to the influences of evolution of the virus such as globalization, trade, settlement, sanitation, other sociodemographic and economic factors, ecologic factors, and environmental factors[4,23,24]. These factors greatly contribute to the easy development and dissemination of the DENV infection, the viral agent, and infected host and vector, thereby cutting across the three walls of epidemiology (environment, susceptible host, and the disease agent)-the epidemiological triad [26].

CHALLENGES IN CONTROL AND PREVENTION OF DENV DISEASE IN NIGERIA

Control of dengue fever in northern Nigeria faces numerous challenges, including limited resources for vector control programs, inadequate healthcare infrastructure, and low awareness of the disease among healthcare providers and the general population. Integrated vector management approaches, such as larval source reduction, use of insecticides, environmental management, and community engagement, are essential for controlling *Aedes* mosquito populations and reducing dengue transmission[8]. Public health education campaigns aimed at promoting personal protective measures, such as using insect repellents, wearing long-sleeved clothing, and sleeping under mosquito nets, are crucial for reducing the risk of dengue infection. Strengthening surveillance systems for early detection and reporting of dengue cases, along with improving access to healthcare services and enhancing capacity for clinical management of dengue patients, are

critical components of dengue prevention and control efforts in northern Nigeria.

SOLUTIONS TO FREQUENT OUTBREAKS OF DENV DISEASE IN NIGERIA

Government/stakeholders' intervention in control of DENV disease

The major way of bringing a remedy to the frequent outbreaks of DENV disease is through vector control. To curb this menace, preventative measures have to be taken against *Aedes* mosquitos which are the vector responsible for the spread of the virus. To achieve this, governmental agencies and other local authorities have roles to play. They must enforce some measures to the public to have an *Aedes*-free environment, this includes monitoring public parks, construction sites, and swampy areas in both rural and urban areas for any possible breeding of the mosquitoes. Plants growing near roadsides or structures, especially those capable of retaining water, require monitoring. Governments should sponsor regular fogging and fumigation using vector repellents in affected areas.

Safe/healthy community practices

Human behavior is the main cause of the spread of DENV. The best way to curb the spread is through the practice of personal and environmental hygiene. The public should ensure that their homes and surroundings do not support the breeding of *Aedes* mosquitoes. This can be achieved through draining and clearing of gutters and domestic water bodies to avoid stagnant water which may serve as a breeding site for *Aedes* mosquitoes; use of insecticides and mosquito repellent; use of insecticide-treated mosquito nets; proper disposal of wastes; and wearing protective clothing to prevent the bite of dengue mosquitoes.

The government must propose innovative solutions to address frequent outbreaks, emphasizing future prevention strategies

In a chemical approach to remedy the spread of DENV, the WHO recommended the use of "Bediocarb" insecticide, which is tested to be effective for the control of *Aedes* mosquitoes. However, in a biological approach to vector control, the use of Wolbachia bacteria has proven to be effective. The Wolbachia bacteria can be introduced to the male *Aedes* mosquitoes, and when they mate with their female counterparts, the eggs will not be hatched, thus preventing the reproduction of new breeds. However, the media also have a role to play in curbing the spread of DENV disease. This may involve raising awareness in schools, hospitals, markets, and other crowded places or social gatherings, and enlightening the public about possible preventive measures to avoid the spread of DENV.

EVALUATING THE RECOMMENDED TAKEDA VACCINE AND PROBLEMS OF ITS ACCEPTABILITY IN NIGERIA

Takeda dengue vaccine (TDV/TAK-003) is a live attenuated tetravalent vaccine which is effective on all the four serotypes of DENV (DENV 1-4) and stimulates various parts of the immune system such as antibodies and immune cells to fight against DENV. The vaccine shots are administered in two doses, subcutaneously, with 3 mo apart[31]. The vaccine is engineered with a live attenuated DENV-2 virus, which is the backbone of the genetic compositions of the other strains of the virus.

Takeda Pharmaceutical Company Limited of Japan manufactures TDV/TAK-003, but the idea of its manufacture was primarily designed by the Division of Vector-Borne Diseases of the Centers for Disease Control and Prevention. The vaccine contains a weakened DENV whose virulence is reduced and when administered, the immune system of the body recognizes the dengue proteins in the weakened viruses as 'foreign' and produces antibodies against them, hence preventing the establishment of the virulent DENV in the body which may cause disease. The vaccine is administered in the form of a subcutaneous injection, which is given two times, with 90-d intervals from the first dose. Presently, the second part of the Phase III Tetravalent Immunization of Dengue Efficacy Study is being conducted in Asia and Latin America, to further test the efficacy and safety of the vaccine[32].

However, the vaccine may encounter challenges in its acceptance in Nigeria, particularly in the northern region, compared to other developed countries[33]. These challenges include a lack of knowledge or misperceptions of vaccine administration by the public; influence of religion and ethnicity; political influence; fear of side effects; lack of faith in vaccines; low level of education; and fear and confusion.

TAKEDA VACCINE AND FUTURE PREVENTION STRATEGIES

Takeda vaccine, endorsed by the WHO's Strategic Advisory Group of Experts on Immunization, is recommended for preventing DENV disease[34,35]. This development represents a significant step forward in the fight against dengue fever. However, comprehensive prevention strategies encompassing vector control, public health education, surveillance, healthcare infrastructure[35,36], and ongoing research are crucial to achieving sustainable dengue control and prevention. By integrating these strategies, we can significantly reduce the global burden of dengue and improve public health outcomes both now and in the future, including in Nigeria.

CONCLUSION

Dengue fever remains a significant public health threat in Nigeria, with recent outbreaks in Sokoto state in northern Nigeria, highlighting the need for effective and continual prevention and control measures. The TDV offers a promising solution to prevent dengue fever, but its acceptance and accessibility in Nigeria, particularly in the northern region, pose challenges. To address these challenges, government intervention, community engagement, and innovative vector control strategies are essential. Strengthening surveillance, increasing public awareness, and enhancing healthcare infrastructure are crucial steps in combating dengue fever in Nigeria. With concerted efforts and collaborative initiatives, Nigeria can mitigate the burden of dengue fever and safeguard public health in the future.

FOOTNOTES

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MINIREVIEWS

Plant-based vaccines against viral hepatitis: A panoptic review

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Abstract

The traditional vaccines against hepatitis have been instrumental in reducing the incidence of some types of viral hepatitis; however, the need for cost-effective, easily distributable, and needle-free vaccine alternatives has led to the exploration of plant-based vaccines. Plant-based techniques offer a promising avenue for producing viral hepatitis vaccines due to their low-cost cultivation, scalability, and the potential for oral administration. This review highlights the successful expression of hepatitis B surface antigens in plants and the subsequent formation of virus-like particles, which have shown immunogenicity in preclinical and clinical trials. The challenges such as achieving sufficient antigen expression levels, ensuring consistent dosing, and navigating regulatory frameworks, are addressed. The review considers the potential of plant-based vaccines to meet the demands of rapid vaccine deployment in response to outbreaks and their role in global immunization strategies, particularly in resource-limited settings. This review underscores the significant strides made in plant molecular farming and the potential of plant-based vaccines to complement existing immunization methods against viral hepatitis.

Key Words: Plant-based therapeutics; Plant vaccines; Edible vaccines; Viral hepatitis; Phytopharmacology and molecular pharming

Core Tip: The review article "Plant-based vaccines against viral hepatitis" explores the innovative approach of using plant-based systems to produce vaccines for viral hepatitis, particularly focusing on hepatitis B and C. Past and recent articles were identified and highlighted using MeSH terminologies on platforms such as PubMed Central, Google Scholar, Scopus, Web of Science, and Research Gate.

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INTRODUCTION

The term "viral hepatitis" denotes liver inflammation caused by a viral infection. Hepatitis viruses are a group of viruses that primarily affect the liver[1]. Hepatitis A, B, C, D, and E are the different types of viral hepatitis, each resulting from a distinct virus. Each type has distinct pathways of transmission, clinical manifestations, geographical distribution, and prevention strategies[2]. Hepatitis B, C, and D are primarily transmitted by contaminated blood or bodily fluids, frequently through sexual contact or sharing needles. Hepatitis A and E are typically caused by ingesting contaminated food or water. Hepatitis B and C are major causes of chronic liver disease and liver cancer, leading to significant morbidity and mortality[3].

The World Health Organization (WHO) estimated in 2019 that 296 million people were living with chronic hepatitis B and 58 million with chronic hepatitis C globally, which causes more than 1.3 million deaths every year[4]. According to a study published in the Indian Journal of Medical Research, the prevalence of hepatitis B surface antigen (HBsAg), indicating chronic hepatitis B infection, in India ranges from 2% to 8% between regions, while hepatitis C virus (HCV) infection in India is estimated to be around 1% to 2%[5,6]. These numbers show how prevalent chronic hepatitis infections are, which, if untreated, can have a permanent adverse impact on health.

To avoid major problems from viral hepatitis, prevention is essential. The WHO's global hepatitis strategy aims to eliminate viral hepatitis as a public health threat by 2030, aiming to reduce new infections by 90% and deaths by 65%[7]. Chronic hepatitis B and C impose a substantial economic burden due to direct healthcare costs and indirect costs such as loss of income and reduced productivity[8]. The WHO and the centers for disease control and prevention have initiated various programs for the surveillance, prevention, and control of hepatitis infections. Despite these efforts, hepatitis remains a global health challenge due to factors such as asymptomatic infections, lack of awareness, and limited access to vaccination and treatment in some regions.

CURRENT HEPATITIS VACCINES

A vital weapon in the fight against viral hepatitis is vaccination. Hepatitis A and B vaccinations, safe sex practices, refraining from sharing needles, and good hygiene are examples of preventive methods. Hepatitis A and B vaccinations are the two most often used hepatitis immunizations[9]. Given its high rate of infection prevention, the hepatitis A vaccination is advised for all children and adults who may be at higher risk. Similarly, highly effective is the hepatitis B vaccination, which is often administered in three or four doses. It is advised for all newborns and people who are more vulnerable[10]. For hepatitis C, there is currently no vaccine available, but research is ongoing to develop one. Vaccination against hepatitis E is also available in some regions where the disease is common, but its use is limited.

These vaccines have significantly reduced the incidence of new infections and the subsequent health complications associated with hepatitis. However, despite the success, there are ongoing challenges related to vaccine formulations, cost, storage requirements, and accessibility that necessitate exploring alternative vaccine strategies[11]. The numerous genotypes and subtypes of the virus that cause hepatitis, their complexity, and the requirement for long-term protection have made developing vaccines against the illness extremely difficult.

Existing hepatitis vaccines and their formulations

Hepatitis B: First-generation vaccines were initially developed from the plasma of chronic hepatitis B virus (HBV) carriers; These vaccines were effective but raised concerns about safety and supply sustainability. Second-generation vaccines are recombinant vaccines produced using yeast cells. They include brands such as Engerix-B and Recombivax HB. These vaccines are safer and more acceptable but still require cold chain storage. Third-generation vaccines include vaccines with adjuvants to enhance immunogenicity, such as the AS04-adjuvanted vaccines used in some hepatitis B and HPV vaccines[12].

Hepatitis A: Havrix and Vaqta are inactivated vaccines requiring refrigeration. They are administered in a two-dose schedule and provide long-lasting immunity[13].

Limitations of current hepatitis vaccines

The cost of hepatitis vaccines can be prohibitive, especially in low-resource settings. Most hepatitis vaccines require refrigeration, which poses a significant challenge in regions lacking adequate infrastructure[14]. The need for cold chain storage limits the reach of these vaccines to remote or underdeveloped areas. The cost and storage requirements compound accessibility issues. Additionally, the need for multiple doses (as in the case of hepatitis A and B vaccines) complicates the completion of the vaccination schedule, particularly in areas with limited healthcare access[15].

Need for alternative vaccine strategies

Developing a vaccine against HCV has been challenging due to the high genetic diversity of the virus and its ability to evade the immune system. There is currently no vaccine available for hepatitis D virus (HDV), and developing a vaccine presents unique challenges due to the dependence of the virus on HBV[16,17].

Given these limitations, there is a pressing need for alternative vaccine strategies. Developing hepatitis vaccinations has been a challenging but necessary effort to decrease the prevalence of viral hepatitis across the world. For managing viral hepatitis, thermostable formulations, single-dose vaccines, vaccines for non-responders, and innovative delivery system vaccines such as plant-based vaccinations provide a viable substitute to conventional vaccine manufacturing techniques[18]. These vaccines are being developed and produced using various technologies, improving their scalability, safety, and efficacy. With this review, we wish to raise awareness of the fact that vaccines derived from plants have a lot of potential for fighting viral hepatitis and will likely be the primary source of easily administered, inexpensive vaccinations in developing countries in the future.

Molecular pharming/farming

Molecular farming, often called biopharming or plant molecular farming, is the process of using plants to produce valuable proteins, peptides, or small molecules for various uses, such as industrial enzymes, medicines, and vaccinations [19,20]. The concept of using plants as bioreactors for vaccine production was pioneered in the late 1980s and has since evolved significantly, offering a viable alternative to traditional vaccine production methods that rely on microbial fermentation or animal cell cultures.

Examples: The synthesis of the anti-cancer drug paclitaxel in the leaves of the Pacific yew tree is among the most well-known examples of pharming. Two further examples are the production of the human immunodeficiency virus-neutralizing antibody 2G12 in tobacco plants and the lactoferrin enzyme in rice[21].

ADVANTAGES OF MOLECULAR FARMING

Cost and scalability of plant-based production systems are two important advantages. Large-scale plant cultivation is feasible in agricultural environments, making it possible to produce significant amounts of target molecules at a reasonable cost[22,23]. The production costs associated with plant-based systems are generally lower than those of traditional systems because they require less energy and fewer high-tech inputs. Plants do not require sterile conditions to the same extent as microbial or mammalian cell cultures, reducing the overall investment and operational costs[24].

Another significant advantage of plant-based vaccines is the potential for oral delivery. Vaccines produced in edible parts of plants could be administered orally, simplifying the vaccination process by eliminating the need for needles and syringes[24]. Plant-based vaccines often exhibit enhanced stability, reducing the need for cold chain logistics[25]. Proteins expressed in plant tissues, especially seeds, can remain stable at room temperature for extended periods. This is crucial for the distribution of vaccines in regions where cold storage facilities are inadequate or non-existent.

The safety profile of plant-based vaccines is potentially superior to traditional vaccines. As plants are free of human pathogens and the risk of contamination with animal viruses and prions is absent, the vaccines produced are inherently safer[26]. Furthermore, plants are very customizable and flexible in their production since they may be readily altered to generate particular drugs or proteins. Tobacco, maize, and rice are among the plant species effectively employed in molecular farming. It is possible to genetically modify these plants to express foreign genes that code for the desired chemical or protein. The target protein is then produced by the plant cells and may be extracted, refined, and used in various ways[27].

Plant-based vaccines allow for the formulation of multi-component vaccines by blending seeds from different transgenic lines expressing various antigens and showing versatility. Plant-based vaccines are biocompatible and provide enhanced mucosal immunity. Public acceptance is also good.

Technologies in developing plant-based vaccines

Plant-based vaccinations against viral hepatitis are made using various methods, each with distinctive advantages and uses. Listed here are a few important technologies:

Genetic engineering: Plant cells are genetically engineered to have genes expressing viral antigens. The primary techniques include codon optimization, subcellular targeting of proteins, and viral vectors. This enables the plants to produce the antigens, which, when delivered as a vaccine, may be utilized to elicit an immune response[19,28].

Transient expression: Transient expression is the process of inserting the antigen-encoding genetic material into the plant for a brief length of time, which causes the antigen to be generated rapidly. One common technique for transient expression is agroinfiltration, where *Agrobacterium tumefaciens*, a bacterium that naturally transfers DNA to plant cells, is used to deliver genetic material into plant tissues[29,30].

Plant transformation techniques: To transfer foreign genes into plants, methods such as biolistic and Agrobacterium-mediated transformation are employed. Stable transformation involves the integration of the desired genes into the plant genome, allowing the plant to express the vaccine antigen continuously over its lifetime and pass the trait to its progeny [31]. This method is used for long-term production of vaccines[32,33].

Downstream processing technologies: Purification and formulation techniques are examples of downstream processing technologies that ensure plant vaccines' safety, stability, and effectiveness. These technological advancements have been made to guarantee that plant-based vaccinations are safe for human use and to satisfy regulatory requirements[34].

Plant breeding and cultivation practices: The development of vaccines based on plants has also benefited from improvements in plant breeding and growing techniques. These techniques provide a consistent and affordable supply of antigens by optimizing the development and output of plants that produce vaccines[33,34].

DEVELOPMENT OF PLANT-BASED HEPATITIS VACCINES

Selection of appropriate antigens

The selection of appropriate hepatitis antigens for plant-based vaccine development involves addressing several challenges, that are informed by immunogenicity, antigenicity, and conservation across viral strains. These challenges are critical to ensure that the resulting vaccine is effective, safe, and capable of providing broad protection against viral strains.

The key challenges in appropriate selection are achieving high immunogenicity and maintaining antigenicity. For example, the envelope protein 2 (E2) glycoprotein of HCV is a primary target for neutralizing antibodies, and its antigenic regions must be conserved in the plant-derived vaccine[35]. Due to the virus's enormous genetic diversity, with seven known genotypes and more than 80 subtypes, and its ability to elude the immune system, producing an HCV vaccine has proven difficult[36]. Selecting antigens that are conserved across these strains is essential for a vaccine that can provide broad protection.

The stability of antigens during processing and storage is essential for maintaining vaccine efficacy. Ensuring that plant-derived antigens are stable and that production methods yield consistent results is challenging. Controlling the dose for self-administered oral vaccines is also difficult and remains to be addressed[37,38].

Studies evaluating immunogenicity, safety and efficacy of plant-based vaccines

Hepatitis A: In recent years, hepatitis A has witnessed promising development in plant-based vaccinations that use genetic engineering and plant transformation methods. Compared to conventional vaccinations, these have benefits, including cost-effectiveness, scalability, and even enhanced stability. Numerous investigations have examined various techniques for creating plant-based vaccinations against hepatitis A, emphasizing antigen expression, effectiveness, and safety.

According to a study by Chuang *et al*[39], the hepatitis A virus (HAV) structural protein virus particle (VP1) was successfully expressed in transgenic tobacco plants. The study results demonstrated that the VP1 protein generated from plants was immunogenic and may induce a specific immune response in mice. This strategy emphasizes the possibility of developing hepatitis A vaccinations using plants.

Mason *et al*[40] used lettuce plants to produce the HAV capsid protein VP1 in a different study. The researchers showed the VP1 protein produced from plants to be structurally and antigenically identical to the natural protein. Mice immunized with the plant-derived VP1 protein developed a particular immunological response, indicating lettuce plants might be used as a platform to produce hepatitis A vaccine.

Hepatitis B: The HBsAg was chosen as the target antigen for vaccination in the process of developing a plant-based hepatitis B vaccine. Using genetic engineering methods, the gene encoding HBsAg was then introduced into the genome of a plant, such as potatoes or tobacco. After that, the plant was grown in carefully regulated environments to manufacture the HBsAg protein.

The efficacy of plant-based hepatitis B vaccinations in preclinical and clinical trials has been shown in several studies. For example, research published in the journal Vaccine in 2005 found that mice could produce a potent immune response to a plant-based hepatitis B vaccine made in tobacco plants[41]. According to another investigation, a plant-based hepatitis B vaccine was successfully produced in lettuce plants[42]. The vaccine also proved to elicit an immunological response in mice.

Hepatitis C: One viable strategy for addressing the worldwide severe health concern of HCV infection is developing a plant-based hepatitis C vaccine. Plants are used as bioreactors to generate HCV antigens, making plant-based vaccinations a scalable and affordable option.

The development of a plant-based hepatitis C vaccine usually entails a few crucial phases. Initially, scientists identify appropriate HCV antigens, such as envelope glycoproteins (E1 and E2), that might trigger a potent immune reaction[43]. Next, using recombinant DNA technology, these antigens are genetically inserted into the genome of a plant, such as a potato, tomato, or tobacco plant. The plant produces the HCV antigens under strict growth conditions, which may then be refined and combined into a vaccine.

Plant-based hepatitis C vaccinations were found to be both feasible and productive in a number of experiments. For instance, research published in 2011 described how tobacco plants could produce HCV envelope glycoproteins successfully. Plant-derived glycoproteins have been demonstrated to elicit particular antibodies against HCV in mice, indicating their immunogenicity. The researchers genetically modified tobacco plants to generate a fusion protein that included many HCV antigens. Mice immunized with the fusion protein produced from plants developed significant humoral and cellular immune responses against HCV[44].

Table 1 Landmark events in the development of hepatitis B and hepatitis C plant-based vaccines

Virus	Plant species	Antigen	Platforms used	Ref.
Hepatitis B	Tobacco	Surface antigen	Transformation	Mason *et al*[46]
	Lettuce, lupin	Surface antigen	Expression	Kapusta *et al*[47]
	Potato	Surface antigen	Genetic engineering	Kong *et al*[48]
	Banana	Surface antigen	Expression	Kumar *et al*[49]
Hepatitis C	Tobacco	Sequence HVR1 from E2 protein	Codon optimization	Piazzolla *et al*[50]
Hepatitis E	Tomato	ORF 2 partial gene	Agroinfiltration	Ma *et al*[51]
	Potato	Capsid protein	Genetic engineering	Maloney *et al*[52]

HVR1: Hypervariable region 1; E2: Envelope protein 2; ORF: Open reading frame.

Hepatitis D: The peculiarity of the virus and its dependence on HBV have made the development of a hepatitis D vaccination difficult. Conventional vaccination strategies targeting the HBsAg have not demonstrated efficacy against HDV. However, in recent years, there have been some promising advancements.

A potential strategy for creating a hepatitis D vaccine is to utilize recombinant DNA technology to generate the hepatitis delta antigen, the HDV antigen, in yeast or mammalian cells[45]. Using this recombinant antigen can then provoke an immunological reaction against HDV.

HIGHLIGHTS OF LANDMARK EVENTS IN THE DEVELOPMENT OF HEPATITIS B AND HEPATITIS C PLANT-BASED VACCINES

These are mentioned in Table 1[46-52].

FUTURE DIRECTIONS

The integration of newer biotechnological techniques, such as transient expression systems and the use of novel plant species, may enhance antigen yield and stability, addressing some of the current limitations. Additionally, establishing international regulatory frameworks and guidelines specific to plant-based vaccine production will be crucial in advancing these vaccines from the laboratory to the bedside.

CONCLUSION

Developing plant-based vaccines against viral hepatitis represents a promising frontier in immunization strategies, particularly for hepatitis B. Over the past decades, significant advancements have been made in plant molecular farming, which has paved the way for producing virus-like particles and other antigenic proteins in plant systems. Recent advancements have also seen the expression of these antigens in different plant systems, such as potatoes and rice, which promise ease of scalability and the potential for edible vaccines, simplifying the administration process and enhancing accessibility. However, despite these promising developments, the transition from laboratory success to clinical and commercial application has been slow. Challenges such as low yields, regulatory hurdles, and the need for consistent and stable expression of antigens in plants remain significant obstacles. Moreover, the immunogenicity and efficacy of these plant-derived vaccines need to be validated in human clinical trials, which are still limited.

FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Cohort Study

Retrospective study evaluating association of colorectal tumors and hepatitis C virus

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Abstract

BACKGROUND

Chronic hepatitis C virus (HCV) has been associated with hepatic and extrahepatic malignancies. Limited studies have shown an association between colorectal adenomas and HCV populations.

AIM

To study the prevalence of colorectal adenomas in patients with HCV compared to the general population and to evaluate if it is an independent risk factor for colorectal adenomas.

METHODS

Patients were divided into HCV and non-HCV based on their HCV RNA titers. Patients with alcoholic liver disease, hepatitis B infection, and inflammatory bowel disease were excluded. Continuous variables were analyzed using the

Mann-Whitney *U* test, and categorical variables using χ^2 with $P < 0.05$ were considered statistically significant. The significant covariates (independent variables) were matched in both groups by propensity score matching, followed by multivariate regression analysis.

RESULTS

Of the 415 patients screened, 109 HCV patients and 97 non-HCV patients with colonoscopy results were included in the study. HCV patients were older, had a smoking history, had less frequent aspirin use, and had a lower body mass index (BMI) ($P < 0.05$). The HCV cohort had a significantly increased number of patients with adenomas (adenoma detection rate of 53.2% *vs* 34%. $P = 0.006$). We performed a propensity-matched multivariate analysis where HCV infection was significantly associated with colorectal adenoma (OR: 2.070, $P = 0.019$).

CONCLUSION

Our study shows a significantly higher rate of adenomas in HCV patients compared to the general population. Prospective studies would help determine if the increase in adenoma detection lowers the risk for colorectal cancer.

Key Words: Hepatitis C; Colon cancer; Adenoma; Polyps; Colonoscopy

Core Tip: There is a paucity of data to suggest the role of chronic hepatitis C virus (HCV) infection and its role with extraintestinal malignancies. Given prior data to suggest HCV is an oncogenic virus, we reviewed its association in patients with colorectal neoplasia.

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INTRODUCTION

Approximately 4. 6 million people are infected by the hepatitis C virus (HCV) in the United States with approximately least 3. 5 million (range 2. 5 million-4. 7 million) currently infected; additional sources suggest that the actual prevalence could be much higher[1-4]. HCV is a multisystemic disease linked to pathological derangements in the body's immunological, cardiovascular, endocrine, respiratory, renal, and neurological systems[5]. In addition to raising the risk of hepatocellular carcinoma (HCC), HCV has been shown to significantly increase the risk of many non-hepatic cancers[6]. Although the HCV genome does not directly combine with the host genome, the proteins generated in response to chronic inflammation have cancer-causing effects, such as inactivating tumor suppressor genes and activating the pro-oncogenes[7,8]. The role of the hepatitis C virus in developing colorectal cancer (CRC) is still unclear due to the limited number of studies examining the association and possible carcinogenic mechanisms.

CRC is the third most common malignancy diagnosed globally, with 1. 8 million new cases reported annually[3,9]. The risk factors for CRC include obesity, a sedentary lifestyle, consumption of red meat and lack of fiber in their diet, excess alcohol consumption, and smoking[10]. More than 15% of cancers worldwide are linked to infective causes; one such is HCV[11]. A retrospective cohort study by Hurtado-Cordovi *et al*[12] discovered that HCV patients had more colorectal polyps than non-HCV patients. However, the results were not statistically significant. Another retrospective study by Su *et al*[13] found that the tumorigenesis within the malignant colonic cells exhibited antibodies to proteins akin to that of hepatocellular carcinoma in patients with chronic hepatitis C increased the risk of CRC on screening colonoscopies in the distal colon. Furthermore, it surprisingly showed that the risk ratio decreased with increasing age, with the highest odds ratio for CRC in HCV patients less than 45 years of age.

In 2020, the United States Preventive Services Task Force (USPSTF), American College of Gastroenterology (ACG), and American Cancer Society (ACS) updated their screening recommendations for CRC from the age of 50 to start at the age of 45 years[14,15]. Since timely intervention in the form of screening colonoscopy can help detect pre-neoplastic or early stages of CRC, it is imperative to understand the relationship between chronic hepatitis C and CRC[16]. Given the lack of literature in this area, we aimed to study the association of chronic hepatitis C and pre-cancerous lesions - colonic adenomatous polyps in patients undergoing screening colonoscopy.

MATERIALS AND METHODS

Study design and participants

This is a retrospective cohort study that included patients who underwent screening colonoscopy in our single-centered community hospital from January 01, 2001, to December 31, 2021. The inclusion criteria were (1) patients aged 18 years and older; (2) patients who underwent screening colonoscopy during the study period as per USPSTF guidelines; (3) they had at least a one-time screening anti-HCV antibody assay done prior to the screening colonoscopy; and (4) the patients had at least 2 or more visits to our primary care clinic in our hospital. The exclusion criteria were (1) patients younger than 18 years of age; (2) patients with incomplete medical records; (3) patients with no biopsy reports when a polyp was found on colonoscopy; (4) patients with concurrent HIV infection, hepatitis B infection, alcoholic liver disease, or high-risk patients like those with inflammatory bowel disease or family history of CRC; (5) patients with documented cirrhosis; and (6) patients with history of colorectal carcinoma.

Exposure and outcomes

Colonoscopy findings were stratified into cohorts based on their results, *i.e.*, hyperplastic, adenomatous, CRC, or normal colonic mucosa. The patients were divided into the HCV group and the non-HCV group based on their HCV RNA viral assay. The HCV group had a positive anti-HCV antibody assay followed by HCV RNA viral titer studies. The non-HCV group were defined as those who had a negative anti-HCV antibody assay. The primary outcome was the detection of adenomatous polyps on colonoscopy. Data collected included age, gender, ethnicity, body mass index (BMI) at the time of the initial clinic visit, family history of CRC, past medical history including diabetes mellitus (DM), medication use which included aspirin use and history of smoking. In patients who tested positive for HCV, their genotype, IL28 gene polymorphism, and HCV viral RNA titer were recorded. Colonoscopy findings included results of the procedure, number of polyps found, if any, type of polyp, size of the polyp, location of polyp, biopsy results of the polyp, and bowel preparation of the colon during the colonoscopy.

Ethical considerations

Institutional review board statement: The study was reviewed and approved by our local Medical Center Institutional Review Board [(Approval No. 2021-035)].

Informed consent statement: Waiver obtained as part of the IRB, owing to retrospective nature of study.

Statistical analyses

The data were subjected to the normalcy test (Shapiro-Wilk test) that showed non-normalcy distribution. Hence, non-parametric tests were employed. The baseline demographic characteristics between the two groups were assessed using χ^2 analysis and Fischer exact test for categorical variables. Mann-Whitney *U* test was used for the study of continuous variables. The association between the covariates and primary endpoint was evaluated using a bivariate logistic regression with the presence of adenomatous polyps as a dependent variable and other significant variables in the univariate analysis as independent variables. The odds ratio (OR) for the presence of adenoma was calculated for qualitative variables. The level of significance was set at 5%. Propensity score matching was used to analyze data further and control for any confounding variables[17]. The propensity score allows one to design and analyze an observational study to mimic some of the characteristics of a randomized controlled trial. HCV was used as the intervention to match the two groups using other covariate variables like age, gender, BMI, aspirin use, smoking, and alcohol use. We matched using the nearest neighbor matching method with a ratio of 1:1 without replacement. After matching, we perform covariate adjustment. Covariate adjustment is most helpful for variables with standardized mean differences more than one. We fit a logistic model, including the covariates in the model, to determine the rate of adenoma detection on colonoscopies (Figure 1).

RESULTS

Patient characteristics

A total of 415 patients were screened, and 206 met our inclusion criteria. 109 HCV patients and 97 non-HCV patients that had colonoscopy results were included in the study. HCV patients were older, with a mean age of 62.73 years, and the non-HCV group with a mean age of 60.20 years (P = 0.026). 44% of HCV patients were females compared to 47.4% of patients in the non-HCV group (P = 0.626). Ethnic groups were equally distributed between both groups. 34.9% of HCV patients were currently smoking at the time of their clinic visit compared to 15.5% of non-HCV patients, and 18.3% of HCV were former smokers compared to 17.5% of non-HCV patients (P = 0.004). HCV patients had a lower BMI with a median BMI of 28 compared to non-HCV patients who had a BMI of 32 (P = 0.001). HCV patients had less frequent aspirin use, with 20.2% on aspirin compared to 36.1% of the non-HCV patients on aspirin (P = 0.011). The HCV group had 24.8% with DM *vs* 32% in the non-HCV group (P = 0.25). The HCV group had a significantly higher number of patients with colonic polyps, 53.2% *vs* 34% in the non-HCV group (P = 0. 006). Further demographics are included in Table 1.

Primary outcome - adenoma detection

We performed bivariate logistic regression with adenoma as a dependent variable and other significant covariates as independent variables (Table 2). HCV patients had an OR of 2.070 for adenomas (P = 0.019). Aspirin use had an OR =

Table 1 Patient demographics, *n* (%)

Variables	HCV (*n* = 109)	Non-HCV (*n* = 97)	OR	95%CI	*P* value
Age (mean ± SD)	62.73 ± 9.14	60.20 ± 7.06			0.026[1]
Female gender	48 (44)	46 (47.4)	0.872	0.504-1.511	0.626[3]
Ethnicity					0.427[3]
Caucasian	81 (74.3)	84 (86.6)	0.447		
Hispanic	18 (16.5)	11 (11.3)	1.546		
Asian	4 (3.7)	2 (2.1)	1.809		
African American	4 (3.7)	0			
Others	2 (1.8)	0			
BMI > 25	28.0 (7)	32.0 (7)			0.001[1,2]
Aspirin intake	22 (20.2)	35 (36.1)	0.448	0.24-0.83	0.011[1,3]
Smoking history			2.31	1.31-4.07	0.004[1,2]
Non-smoker	51 (46.8)	65 (67)			
Current smoker	38 (34.9)	15 (15.5)			
Former smoker	20 (18.3)	17 (17.5)			
Total pack year, median (IQR)	25 (7)	25 (7)			0.757[2]
DM	27 (24.8)	31 (32)	0.872	0.50-1.51	0.25[3]
Adenomatous polyps	58 (53.2)	33 (34)	2.206	1.25-3.876	0.006[1,3]
Bowel preparation					0.148[3]
Good	90 (82.6)	88 (90.7)	0.484		
Fair	11 (10.1)	7 (7.2)	1.443		
Poor	8 (7.3)	2 (2.1)	3.762		

[1]Statistically Significant.
[2]Mann Whitney *U* test.
[3]χ^2 test.
HCV: Hepatitis C virus; BMI: Body mass index; OR: Odds ratio; IQR: Interquartile range; CI: Confidence interval; DM: Diabetes mellitus.

0.513 (P = 0.065).

In terms of outcome, the analysis showed that patients with HCV showed a more significant number of polyps (88) as compared to patients with non-HCV (58). Other variables, such as location, size, and histopathology, were also analyzed in Table 3. Tubular and hyperplastic polyps showed more propensity for HCV (58 and 22, respectively) compared to non-Hep C, where the tubular adenoma was 38 and hyperplastic was 18. Our data consisted of patients who were predominantly HCV genotype 1a (63.7%, P = 0.8). Other genotypes included were 1b (10.3%), 2b (5.1%), 2 (13.7%), 4 (6.8%), and 6 (0%), as depicted in Table 4. Patients who were HCV-positive were tested for IL28 polymorphisms (interleukin 28). 56% had CT polymorphism, 33.9% had CC, and TT 10.1%, as shown in Table 5.

We performed a 1:1 propensity score matching with HCV as the intervention and control for other covariates between the two groups. There were 97 matches between the cases and the control arm. Twelve were excluded as they were unmatched, and 0 were discarded. After matching, covariate adjustment was made, and we repeated the multivariate analysis where hepatitis C had an OR: 2.069, P = 0.019 for colonic adenoma. Age had an OR = 1.043, P = 0.0295, and aspirin use had an OR = 0.387, P = 0.0116 for colonic adenoma detection (Table 6).

DISCUSSION

Viruses are infective particles implicated in various disease processes, ranging from simple upper respiratory tract infections to malignancies. In the United States, hepatitis C is the most common chronic viral blood-borne infection[18]. Globally, an estimated 58 million people have chronic hepatitis C infection, with about 1.5 million new cases were detected yearly. HCV is associated with a variety of diseases, including autoimmune vasculitis, cryoglobulinemia, clotting disorders, lymphoproliferative neoplasm, and solid tumors[19,20]. The relationship between HCC and chronic HCV has been well established; however, there is limited literature about this infection's effects on the carcinogenesis of

Table 2 Bivariate logistic regression for colonic adenoma

Patient characteristics	*P* value	OR	95%CI for OR	
			Lower	Upper
Age (> median)	0.56	1.18	0.66	2.12
Presence of HCV	0.019[1]	2.07	1.12	3.81
Aspirin intake	0.06	0.51	0.25	1.04
Smoking history	0.49	1.22	0.67	2.23
Female gender	0.86	1.05	0.58	1.88
Diabetes mellitus	0.52	1.24	0.63	2.45

[1]Statistically significant.
HCV: Hepatitis C virus; OR: Odds ratio.

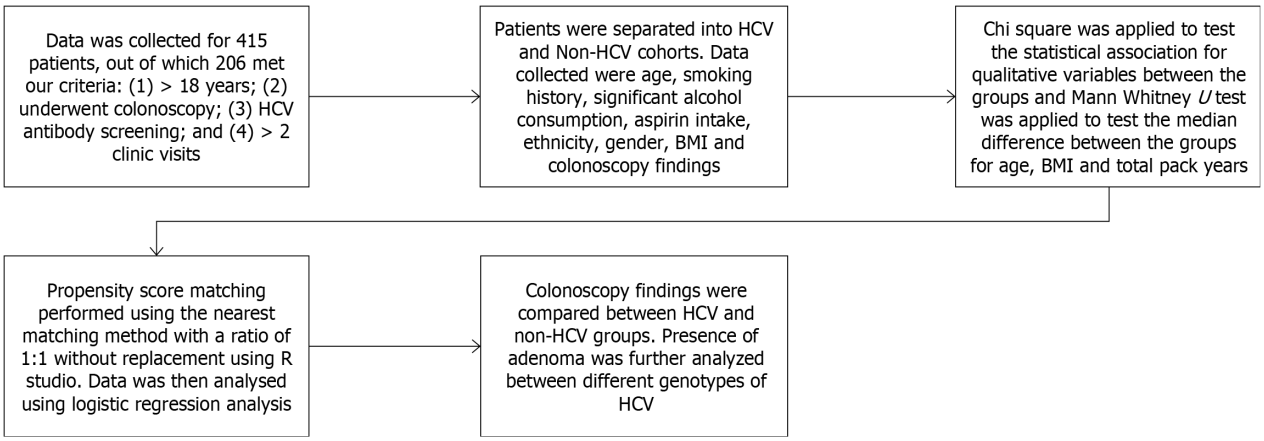

Figure 1 Summary of methodology. HCV: Hepatitis C virus; BMI: Body mass index.

other common malignancies, namely CRC[7,21]. Most colon tumors arise from adenomatous polyps; it is not well known if HCV influences the growth and development of these precancerous lesions, thereby increasing the risk of CRC[22,23].

There have been a handful of retrospective studies that have investigated HCV and its association with CRC. In Allison *et al*[24], an increased incidence of rectal cancer was found in patients with chronic HCV infection (OR: 2.1, 95%CI: 1.3–2.8), but CRC incidence rate did not increase in chronic HCV-infected patients (OR: 0.4, 95%CI: 0.3–0.6). Another study showed that HCV infection is a separate risk factor for advanced neoplasia and hyperplastic polyps discovered during screening colonoscopies[25]. The total number of adenomas was higher in the HCV group as compared to the non-HCV group; however, not statistically significant (0.69 *vs* 0.58 per patient; $P > 0.05$). According to a 2018 systematic review article, young adults with hepatitis C have a higher risk of developing CRC, which was associated with worse outcomes[26].

To understand this association better, we conducted a retrospective cohort study on 415 consecutive patients who underwent screening colonoscopy that were previously screened for hepatitis C. The patients were then divided into two groups, those with chronic hepatitis C (HCV group) and those without (non-HCV group). Patients in the HCV group were older, smoked more often, had a lower BMI, and used aspirin less frequently than the non-HCV group. Ethnicity groups, female gender and comorbidities like DM were equally distributed between both groups. HCV patients had a higher risk of polyps that were detected on their screening colonoscopy than non-HCV patients. We found that HCV patients had higher odds of colonic adenomatous polyps than non-HCV patients on logistic regression analysis.

Given that pre-cancerous lesions and cancer have multiple risk factors, many of which are unmodifiable, like age, gender, and ethnicity. There are also certain modifiable factors like smoking, aspirin intake, higher BMI, and chronic hepatitis C, which influence the risk for CRC. As these risk factors could impact our results and act as confounders, we applied propensity score matching to control for these potential confounders. The propensity score was estimated using a logistic regression model, in which treatment status was regressed on observed baseline demographics. HCV status was used as intervention arm and the propensity score was calculated. Following this, 1:1 propensity score matching was conducted. Whereby, matched sets of treated and untreated patients who share a similar value of the propensity score are reanalyzed. After covariate adjustment, we repeated the logistic regression analysis, which also showed that patients with HCV have 1. 89 higher odds of colonic adenoma than non-HCV patients.

Table 3 Polyp characteristics and location in hepatitis C virus patients and non-hepatitis C virus patients, *n* (%)

Polyp characteristics	HCV	Non-HCV
Number of polyps	88 (60.27)	58 (39.72)
Polyp size < 5 mm	59 (67.04)	38 (65.51)
Range of polyp size (mm)	1-30	1-18
Histopathology of lesions:		
Tubular Adenoma	58 (74.6)	38 (74.4)
Hyperplastic	28 (23.7)	18 (23.1)
Cancer	2 (1.7)	0
Inflammatory	0	2 (2.5)
Location:		
Right colon	29 (32.9)	14 (24.1)
Left colon	17 (19.3)	9 (15.5)
Transverse colon	14 (15.9)	14 (24.1)
Sigmoid colon	20 (22.7)	8 (13.7)
Rectum	8 (9.09)	13 (22.4)

HCV: Hepatitis C virus.

Table 4 Genotype distribution among hepatitis C virus patients, *n* (%)

HCV genotype	With adenoma (*n* = 58)	Without adenoma (*n* = 51)
1a	37 (63.7)	28 (54.9)
1b	6 (10.3)	11 (21.5)
2b	3 (5.1)	2 (3.9)
3	8 (13.7)	9 (17.6)
4	4 (6.8)	0
6	0	1 (1.9)

HCV: Hepatitis C virus.

Table 5 *Il28* gene polymorphism in hepatitis C virus patients, *n* (%)

Il28 gene polymorphism	HCV patients
CC	37 (33.9)
CT	61 (56)
TT	11 (10.1)

HCV: Hepatitis C virus.

In 2020, CDC and USPTF recommended a universal one-time HCV screening for the population aged 18 years and above, irrespective of their year of birth[26]. Given the updated screening recommendation, we expect the incidence of new acute and chronic hep C patients to increase steadily over the next few years. Furthermore, HCV affects multiple generations, the highest among two age groups: 20–39 and 55–70 years[20]. The USPSTF, ACG, and ACS recently recommended screening for colorectal cancer starting at age 45 years in average-risk individuals instead of 50 years[26]. Considering our study showing an increased association of colorectal adenoma in HCV patients, we find the current guidelines for CRC screening would not be able to adequately screen patients aged 20-39 with HCV. Even though the HCV group was older, with a mean age of 62 years, about 85% of excluded patients were chronic hepatitis C patients

Table 6 Covariate adjustment for colonic adenoma post propensity score matching

Patient characteristics	OR	*P* value	Standard error
Hepatitis C	1.89	0.0314[1]	0.33
Age	1.04	0.0295[1]	0.01
Female gender	1.02	0.9428	0.31
BMI	1.00	0.9164	0.02
Aspirin intake	0.38	0.0116[1]	0.37
Smoking history	0.95	0.80	0.20

[1]Statistically significant.
OR: Odds ratio; BMI: Body mass index.

without any colonoscopy results, as they were < 45 years of age and hadn't undergone a colonoscopy yet. We think the true prevalence of colonic adenomas is underestimated in our study and most other studies due to this fact.

Limitations

One of the limitations of our study is the small sample size. Since the study is single-centered and retrospective, the generalizability of the study is limited. Furthermore, due consideration was given to possible confounding factors; we used special statistical analyses like propensity score, 1:1 matching, and covariate adjustment. Another limitation of our study is that we did not document if a patient underwent > 1 colonoscopy. As most of our patients were younger than the age for CRC screening, they mostly had only a single colonoscopy. We also did not document the temporal relationship of HCV treatment with the timing of their colonoscopy. Since our study period extended from 2001 to 2021, there have been varied treatment regimens for HCV. This could be an area of future studies to investigate if treatment with novel direct-acting antivirals reduces the risk of association between HCV and colonic adenomas by documenting serial colonoscopies.

We did not analyze data for possible correlation between HCV viral load, specific genotype, Il28 gene polymorphism of HCV, and risk of adenoma/ advanced neoplasia. We postulate that the cumulative effect of duration and HCV viral load increases the risk of adenoma production. HCV patients with chronically elevated viral loads for extended periods could be at highest risk of adenoma and colorectal carcinogenesis. Prospective studies could investigate if the treatment of hepatitis C reverses the risk of colorectal adenomas.

Adenoma detection rate is variable depending on the experience of the endoscopist, withdrawal time, first time endoscopy for the patient. We were unable to obtain this information to sub-stratify our findings.

CONCLUSION

Our study shows a significantly higher rate of colonic adenomas in chronic hepatitis C patients. On multivariate analysis with and without propensity score matching, HCV infection was found to be an independent risk factor for colorectal adenoma. Current guidelines do not recommend earlier screening for CRC for patients with chronic hepatitis C. Prospective studies are required to assess if treatment of HCV leads to lower adenoma detection and if early adenoma detection in these patients prevents the development of CRC in the future.

FOOTNOTES

Author contributions: Yekula A conceived the idea for the study; Gogtay M, Singh Y, and Yekula A designed and undertook the literature review, collected data; Gogtay M and Bullappa A performed the statistical analysis and interpreted the data; Gogtay M, Singh Y, and Yadukumar L wrote the first draft of the manuscript; Gogtay M, Singh Y, Yadukumar L, Soni A, and Abraham GM 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

Retrospective Cohort Study

Human immunodeficiency virus cascade–continuum of care stages and outcomes in a hospital in southern Brazil

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Abstract

BACKGROUND

The human immunodeficiency virus (HIV) continuum of care cascade illustrates the 90-90-90 goals defined by the Joint United Nations Program on HIV/acquired immunodeficiency syndrome (UNAIDS). The care cascade includes the following five steps: Diagnosis, linkage to care, retention in care, adherence to antiretroviral therapy (ART), and viral suppression.

AIM

To elaborate the HIV cascade of patients diagnosed with HIV at the Nossa Senhora da Conceição Hospital (HNSC) and to determine possible local causes for the loss of patients between each step of the cascade.

METHODS

This retrospective cohort study included patients diagnosed with HIV infection

from January 1, 2015 to December 31, 2016 and followed up until July 31, 2019. The data were analyzed by IBM SPSS software version 25, and Poisson regression with simple robust variance was used to analyze variables in relation to each step of the cascade. Variables with $P < 0.20$ were included in multivariable analysis, and $P < 0.05$ was considered significant. Pearson's χ^2 test was used to compare the groups of patients followed up at the HNSC and those followed up at other sites.

RESULTS

The results were lower than those expected by the UNAIDS, with 94% of patients linked, 91% retained, 81% adhering to ART, and 84% in viral suppression. Age and site of follow-up were the variables with the highest statistical significance. A comparison showed that the cascade of patients from the HNSC had superior results than outpatients, with a significant difference in the last step of the cascade.

CONCLUSION

The specialized and continued care provided at the HNSC was associated with better results and was closer to the goals set by the UNAIDS. The development of the HIV cascade using local data allowed for the stratification and evaluation of risk factors associated with the losses occurring between each step of the cascade.

Key Words: Cascade; Continuum care; Human immunodeficiency virus; Antiretroviral therapy; Adherence

Core Tip: The capital of southern Brazil, Porto Alegre, has the highest acquired immunodeficiency syndrome (AIDS)-related mortality rate. These data demonstrate that human immunodeficiency virus (HIV)/AIDS is an important public health problem in the city, and it is important to carry out studies to improve indicators. The HIV cascade was adopted as a portrait of implemented public policies. That is why we decided to carry out this study with the objective of identifying the steps and results of continuity of care in patients diagnosed with HIV infection in a hospital located in Porto Alegre. The development of the HIV cascade using local data allowed for the stratification and assessment of risk factors associated with losses occurring between each stage of the cascade, to develop new strategies aimed at achieving the 90-90-90 target in future assessments.

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INTRODUCTION

Antiretroviral therapy (ART) has definitively changed the course of the human immunodeficiency virus (HIV) epidemic, transforming it into a chronic disease, with a life expectancy similar to that of an adult without HIV infection[1,2]. In contrast, the Joint United Nations Program on HIV/acquired immunodeficiency syndrome (UNAIDS) estimates that half of all people living with HIV (PLHIV) are unaware of their diagnosis and approximately 22 million people aware of the diagnosis have no access to ART, despite advances in awareness-raising campaigns about the disease[3]. These data show gaps in PLHIV care that result in continued viral transmission[1]. Therefore, at the end of 2013, the UNAIDS established the 90-90-90 goals, which consist of a HIV continuum of care cascade that includes the following five steps: Diagnosis, linkage to care, retention in care, adherence to ART, and viral load (HIV-VL) suppression[4,5]. This HIV cascade has been adopted as a snapshot of the public policies implemented[6].

Brazil was one of the first countries in Latin America to adopt the goals set by the UNAIDS[7]. Estimates show that in 2021, approximately 960000 people were infected with HIV in Brazil; among all the individuals infected with HIV, 89% were aware of the diagnosis, 82% were linked to some health service, 76% retained their respective health services, 73% were on ART, and 65% had achieved HIV-VL suppression. These estimates were higher than those in previous years[7]. Unawareness of HIV infection, sexual behavior with low HIV risk perception, and low adherence to ART with HIV-VL recovery were the main causes of HIV infection in Brazil[8].

The Nossa Senhora da Conceição Hospital (HNSC) is a tertiary hospital located in Porto Alegre, in the state of Rio Grande do Sul (RS), Brazil, a city that ranked second in 2021 among the Brazilian capitals, with the highest AIDS detection rate[9]. In the same year, Porto Alegre also obtained the highest mortality rate, which was five-fold higher than the national rate and, the highest HIV detection rate in pregnant women, which was six-fold higher than the national rate [9]. The HNSC is a reference hospital for the care of PLHIV, with inpatient and outpatient lines of care for children, adolescents, adults, and pregnant women[10]. This study aimed to identify the characteristics of the patients diagnosed with HIV in the HNSC and recognize the continuum of care stages and outcomes in these patients through elaboration of the HIV cascade with local data. Thus, the HIV cascade was developed with local data to determine possible local causes

for the loss of patients between each step of the cascade. As HNSC is a reference in public service in the region, the hypothesis is that the data would be very close to the UNAIDS target, despite the high mortality rate in the city.

MATERIALS AND METHODS

This retrospective cohort study included patients diagnosed with HIV infection from January 1, 2015 to December 31, 2016 and followed up until July 31, 2019. Patients aged 18 years or older, with a positive anti-HIV test (routine serology or screening), who were admitted or received outpatient care at the HNSC during the period of analysis, were included in the study. The exclusion criteria included the following: An HIV diagnosis before 2015 or after 2016 and missing data in the HNSC electronic medical records. Patients who died were included in the first analysis to identify the characteristics of the patients but were not considered in the cascade analysis. Data from the HNSC laboratory and HNSC electronic medical records were analyzed and linked with those from the Laboratory Test Control System and Medication Logistic Control System. Thereafter, a comparison was made between patients followed up after hospital discharge at the infectious disease outpatient clinic of the HNSC and at other sites (other cities or health institutions).

The requirements for each step of the cascade were subjectively defined based on the criteria of the Brazilian Ministry of Health. The first step included the number of people diagnosed with HIV infection in the period, always illustrated with the percentage of 100%. The second step included patients linked to some specialized service; after diagnosis, they underwent at least one CD4 + T-cell count or HIV-VL test. The third step of the cascade showed the number of users retained in the service, *i.e.*, those who underwent at least two HIV-VL tests or two CD4 + T-cell counts, regardless of the period between them. The fourth step registered the number of patients on ART and who were still collecting their medications in 2019 (last analysis period). The fifth step included adherent patients to ART with an undetectable HIV-VL (< 50 copies/mL) at the last test. After analysis, five exposure columns were constructed to define the HIV cascade of all patients involved in the study who did not die. The percentages were calculated both in relation to the first and previous steps.

The analyses were performed using IBM SPSS software version 25. Poisson regression with simple robust variance was used to estimate the incidence ratio (IR) at a 95%CI for each variable, including sex, age group, race, education, city of residence, and follow-up site, in relation to each step of the cascade: Linked, retained, on ART, and HIV-VL suppressed. All variables with $P < 0.20$ in simple analyses were included in the multivariable model. Adjustments were made only for the last two steps (on ART and undetectable HIV-VL), and only variables with $P < 0.05$ were considered significant.

Two HIV cascades were also created, discriminating patients by follow-up location after HIV diagnosis at HNSC and hospital discharge. Each step of the HIV cascades of patients followed up at the HNSC and at other sites, as well as the sociodemographic characteristics of these groups of patients, were compared using the Pearson's χ^2 test, and results with $P < 0.05$ were considered significant. This study was approved by the Research Ethics Committee of the Hospital Group and the requirement for informed consent was waived upon commitment to patient confidentiality.

RESULTS

First analysis–characteristics of patients

The study included 629 patients diagnosed with HIV infection in 2015 and 2016. The profile of the participants by sociodemographic characteristics showed that 440 (70%) were white, 339 (54%) were women with a mean age of 40 years at diagnosis, 440 (70%) had completed elementary school, and 421 (67%) were from Porto Alegre. According to the medical records, 150 (24%) had comorbidities such as hypertension (12%), diabetes (4%), and asthma (3.5%), the most frequent illnesses among all the patients.

Moreover, around 471 (75%) of the patients had been hospitalized at least once in the HNSC after being diagnosed with HIV infection. The following causes of hospitalization are highlighted: HIV infection (22%), normal delivery or cesarean section (18%), and tuberculosis (11%). Opportunistic infections occurred in 164 (35%) of hospitalized patients, mostly tuberculosis (34%), toxoplasmosis (20%), pneumocystosis (14%), cytomegalovirus infection (12%), and cryptococcosis (12%). Another frequent AIDS-defining disease was lymphoma (6%). Of the patients diagnosed with opportunistic infections, 137 (84%) had a CD4 + T-cell count of < 200 cells/mm^3.

Following an HIV diagnosis, 584 (93%) of patients underwent at least one CD4 + T-cell count and/or HIV-VL test. The time between diagnosis and the first CD4 + T-cell count was, for most patients, up to 30 days (75%); cumulatively, 94% had their samples collected within 6 months. Of the first CD4 + T-cell counts, 41% of patients had counts < 200 cells/mm^3, 32% between 201 and 500 cells/mm^3, and 27% > 500 cells/mm^3. There were no differences in the first CD4 + T-cell collection between inpatients and outpatients. Moreover, 461 (79%) of the patients had undergone two or more CD4 + T-cell count tests, of whom 15% had a final CD4 + T-cell count of < 200 cells/mm^3, 30% between 201 and 500 cells/mm^3, and 55% of > 500 cells/mm^3. Of the patients with a final CD4 + T-cell count of > 500 cells/mm^3, 79% were ART adherent; however, of those who had a final CD4 + T-cell count of < 200 cells/mm^3, only 37% were ART adherent.

ART medication was collected at least once by 534 (85%) of patients. The first ART prescription after an HIV diagnosis occurred within 30 days in 51% of cases, within 6 months in 86%, and within 1 year in 93%. The main regimen was tenofovir, lamivudine, and efavirenz (TDF/3TC/EFZ), corresponding to the treatment used in 62% of the patients; this was followed by tenofovir and lamivudine (TDF/3TC) + atazanavir and ritonavir (ATV/r), with 16% of the patients receiving this combination, and TDF/3TC + dolutegravir, with this being prescribed to only 5.2%. Approximately 120

patients had a history of pregnancy in the period and were treated with TDF/3TC/ATV/r (32%), TDF/3TC/EFZ (29%), and AZT/3TC + lopinavir and ritonavir (LPV/r) (26%). The analysis indicates that 69% of patients on ART did not switch regimens and 22% switched regimens only once. In addition, 95 patients (15%) died and were not included in further cascade analysis. Among these patients, the main cause of death was sepsis (42%), with 57% being diagnosed with opportunistic infections and 72% presenting a CD4 + T-cell count of < 200 cells/mm^3.

HIV cascade

Excluding deaths, 534 patients were selected for the cascade analysis, 500 (94%) were linked to the service, 455 (85%) were retained in care, 368 (69%) were on ART, and 308 (58%) achieved HIV-VL suppression (Figure 1). If the previous column was considered the denominator, the percentages would be as follows: 94% linked, 91% retained, 81% on ART, and 84% HIV-VL suppressed. Patient characteristics (sex, age, race and education, city of origin, and follow-up site) are listed in Table 1. Patients included in the cascade were predominantly women, white, aged 30–49 years, living in Porto Alegre, and with elementary school education. Of these, only 52% remained under follow-up at the HNSC.

Only the variables education, in the linked step, and sex in the retained step, presented a $P < 0.20$; thus, they were included in the multivariable analysis. Regarding the column on ART in the univariate analyses, there was a significant difference ($P < 0.20$) for the variables sex, age group at diagnosis, education, race, place of residence, and follow-up site. Being a woman, not white, aged 18–30 and 31-49 years, not having complete higher education, and being followed up outside the HNSC were considered risk factors for non-adherence to ART. However, living in the metropolitan region was considered a protective factor for adherence to ART. In the multivariable analysis (Table 2), only the age groups 18–30 and 31–49 years were considered significant risk factors for non-adherence to ART ($P < 0.05$).

Regarding the data in the last column, being aged 18–49 years, not having higher education, not being white, and not being followed up at the HNSC were considered risk factors for not achieving HIV-VL suppression ($P < 0.20$). In the multivariable analysis, follow-up at the HNSC was considered a protective factor for HIV-VL suppression ($P < 0.05$). Similarly, in adherence to ART, being aged 18–30 and 31-49 years alone was considered a risk factor for not presenting an undetectable HIV-VL ($P < 0.05$). It was not possible to analyze the use of drugs, consumption of alcohol, and smoking habits as variables of association with cascade steps due to lack of data in the medical records.

HIV cascade HNSC vs other sites

Of the 534 patients included in the HIV cascade, 78 had no outpatient appointments documented. In addition, 236 remained at the HNSC and 220 chose to have appointments at other sites. At the HNSC, 225 (95%) were retained, 197 (83%) adhered to ART, and 172 (73%) achieved HIV-VL suppression. Of the outpatients, 209 (95%) were retained, 169 (77%) adhered to ART, and 135 (61%) had an undetectable HIV-VL, as shown in Figure 2. The last column, comprising patients who achieved HIV-VL suppression, showed a significant difference, with $P = 0.009$.

The comparison between the characteristics of patients followed up at the HNSC and those followed up at other sites showed similar results with respect to sex, age group, race, education, and history of hospitalization (Table 3). There was also no difference between the groups regarding initial and final CD4 + T-cell counts, number of sample collections for CD4 + T-cell count and HIV-VL, and number of ART regimens. Only the city of residence and time of ART initiation after HIV diagnosis showed significant differences ($P < 0.05$) between the groups.

Of the patients followed up at the HNSC, 66.2% had their first appointment within 6 months and 81.3% within 1 year. After diagnosis, 42% of patients had two to four appointments in the period analyzed and 43% had five to eight, equivalent to an average of two appointments per year, as indicated by the Ministry of Health. According to these recommendations, 50% of the patients who achieved HIV-VL suppression had five to eight appointments. Similarly, 70% of the patients who achieved no HIV-VL suppression had fewer than five appointments.

DISCUSSION

The first step in the HIV continuum of care cascade is diagnosis, and the major challenge is to make this diagnosis at the beginning of the infection[11]. In this study, the number of patients who had HIV infection and were unaware of it was not calculated, which is the first column defined by the UNAIDS. Early diagnosis implies reduced morbidity, mortality, costs, and transmission by individuals with unknown serological status, which influences all subsequent steps in the HIV continuum of care cascade[12,13]. To avoid this, it would be essential to increase access to anti-HIV testing, based on national recommendations and local epidemiology[12,13]. The HNSC has implemented a flow of serological tests for infectious diseases at admission, and many of the HIV diagnoses are made in patients seeking care for symptoms unrelated to the disease. This can be verified in the causes for hospital admission, with almost 80% of cases not being related to HIV infection.

The characteristics of the patients who participated in this study differed from the epidemiological data from Brazil and RS regarding the predominance of women, possibly because women more frequently seek care from health services. The dominant age range in this study was 30–49 years, corroborating the age range in the RS (35–39 years) and Brazil (25–39 years)[14,15]. However, there was a difference in this study's data, which showed a predominance of white people, and the national data (which showed a predominance of black people). This is justified because most of the population in RS is white due to European colonization[14,15]. The HNSC is a hospital that provides 100% care through the Unified Health System, with a higher prevalence of patients with lower purchasing power and low educational level. State and national data also report that most patients have a low educational level[14,15]. It was not possible to compare the infection rate between heterosexual or homosexual individuals due to lack of data in the medical records.

Table 1 Analysis of variables with absolute number and relative percentage and Poisson regression with robust simple variance of each variable in relation to the steps of the cascade of patients diagnosed with human immunodeficiency virus in the Nossa Senhora da Conceição Hospital, *n* (%)/95%CI

	Total	Linked	*P* value	Retained	*P* value	On ART	*P* value	HIV-VL suppressed	*P* value
Total	534	500 (93.6)		455 (85.2)		368 (68.9)		308 (57.6)	
Gender									
Female	303 (56.7)	284 (93.7)	0.917	264 (87.1)	0.161	198 (65.3)	0.039	162 (53.5)	0.023
		1.00 (0.95-1.04)		1.05 (0.97-1.13)		0.88 (0.79-0.99)		0.84 (0.73-0.97)	
Male	231 (43.3)	216 (93.5)		191 (82,7)		170 (73,6)		146 (63,2)	
		1.00		1.00		1.00		1.00	
Age (yr)									
18-29	154 (28.8)	148 (96.1)	0.252	129 (83.8)	0.420	92 (59.7)	0.000	70 (45.5)	0.000
		1.03 (0.97-1.10)		0.96 (0.86-1.06)		0.73 (0.63-0.86)		0.63 (0.51-0.78)	
30-49	270 (50.6)	250 (92.6)	0.964	230 (85.2)	0.585	187 (69.3)	0.012	159 (58.9)	0.011
		0.99 (0.93-1.06)		0.97 (0.89-1.06)		0.85 (0.75-0.96)		0.82 (0.70-0.95)	
> 50	110 (20.6)	102 (92.7)		96 (87.3)		89 (80.9)		79 (71.8)	
		1.0		1.0		1.0		1.0	
Race									
White	374 (70.0)	348 (93.0)		319 (85.3)		266 (71.1)		255 (60.2)	
		1.0		1.0		1.0		1.0	
Not white	160 (30.0)	152 (95.0)	0.367	136 (85.0)	0.930	102 (63.7)	0.108	83 (51.9)	0.089
		1.02 (0.97-1.06)		0.99 (0.92-1.07)		0.89 (0.78-1.02)		0.86 (0.72-1.02)	
Education									
Illiterate	16 (3.0)	12 (75)	0.188	11 (68.8)	0.103	9 (56.3)	0.031	7 (43.8)	0.064
		0.80 (0.58-1.11)		0.74 (0.51-1.06)		0.60 (0.38-0.95)		0.55 (0.30-1.03)	
Elementary School	367 (68.7)	346 (94.3)	0.840	310 (84.5)	0.221	246 (67.0)	0.000	202 (55.0)	0.016
		1.01 (0.87-1.17)		0.91 (0.78-1.05)		0.72 (0.61-0.84)		0.70 (0.52-0.93)	
High School	136 (25.5)	128 (94.1)	0.861	120 (88.2)	0.526	99 (72.8)	0.007	87 (64.0)	0.181
		1.01 (0.87-1.17)		0.95 (0.81-1.11)		0.78 (0.65-0.93)		0.81 (0.60-1.10)	
University Education	14 (2.6)	13 (92.9)		13 (92.9)		13 (92.9)		11 (78.6)	
		1.0		1.0		1.0		1.0	
City of origin									
Porto Alegre	370 (69.3)	350 (94.6)		312 (84.3)		249 (67.3)		210 (56.8)	
		1.0		1.0		1.0		1.0	
Metropolitan region	128 (24.0)	118 (92.2)	0.367	112 (87.5)	0.358	94 (73.4)	0.175	79 (61.7)	0.313
		0.97(0.92-1.03)		1.03 (0.95-1.12)		1.09 (0.96-1.23)		1.08 (0.92-1.28)	
Countryside	36 (6.7)	32 (88.9)	0.302	31 (86.1)	0.766	25 (69.4)	0.787	19 (52.8)	0.658

		0.94 (0.83-1.05)		1.02 (0.88-1.17)		1.03 (0.82-1.29)		0.93 (0.67-1.28)	
Follow-up site									
HNSC	236 (51.8)	236 (100)		225 (95.3)		197 (83.5)		172 (72.9)	
		1.0		1.0		1.0		1.0	
External	220 (48.2)	219 (99.5)	0.317	209 (95.0)	0.866	169 (76.8)	0.07	135 (61.4)	0.010
		0.99 (0.98-1.00)		0.99 (0.95-1.03)		0.92 (0.83-1.00)		0.84 (0.73-0.95)	

P < 0.20 considered significant. ART: Antiretroviral therapy; HIV-VL: Human immunodeficiency virus-viral load; HNSC: Nossa Senhora da Conceição Hospital.

Table 2 Poisson regression analysis with multivariable robust variance in relation to the steps on antiretroviral therapy and human immunodeficiency virus-viral load suppressed of patients diagnosed with human immunodeficiency virus in the Nossa Senhora da Conceição Hospital, 95%CI

	On ART	*P* value	HIV-VL suppressed	*P* value
Gender				
Female	0.93 (0.85-1.02)	0.163	0.90 (0.80-1.02)	0.126
Male	1.00		1.00	
Age (yr)				
18-29	0.79 (0.69-0.90)	0.000	0.66 (0.54-0.80)	0.000
30-49	0.90 (0.82-0.99)	0.041	0.87 (0.76-0.99)	0.044
> 50	1.0		1.0	
Race				
White	1.0		1.0	
Not White	0.94 (0.85-1.05)	0.325	0.91 (0.78-1.05)	0.215
Education				
Illiterate	0.98 (0.75-1.28)	0.886	1.03 (0.68-1.55)	0.881
Elementary School	0.86 (0.73-1.00)	0.060	0.86 (0.66-1.14)	0.311
High School	0.93 (0.78-1.10)	0.415	1.03 (0.78-1.37)	0.794
University Education	1.0		1.0	
City of origin				
Porto Alegre	0.96 (0.80-1.15)	0.693		
Metropolitan region	1.06 (0.88-1.27)	0.534		
Countryside	1.0			
Follow-up site				
HNSC	1.09 (0.99-1.20)	0.055	1.18 (1.03-1.34)	0.011
External	1.0		1.0	

P < 0.05 considered significant. ART: Antiretroviral therapy; HIV-VL: Human immunodeficiency virus-viral load; HNSC: Nossa Senhora da Conceição Hospital.

A late diagnosis is defined by an HIV diagnosis with a first CD4 + T-cell count < 200 cells/mm³, which indicates late presentation to the health system and failure to access a diagnosis[12,16]. Although most patients had this test when their counts were > 200 cells/mm³ at diagnosis, 41% were diagnosed late, with their initial CD4 + T-cell count < 200 cells/mm³. CD4 + T-cell counts < 200 cells/mm³ were indicative not only of opportunistic infections but also death. There is a trend in RS and in Brazil toward a reduced late diagnosis rate, and this may be one of the contributions to a reduced coefficient of mortality by HIV/AIDS[14,15]. In the state of RS, only 23% of people diagnosed with HIV infection had a

Table 3 Comparison between patients who underwent follow-up at the Nossa Senhora da Conceição Hospital and external institutions using Pearson's χ^2 test, *n* (%)

	HNSC	External	*P* value
Total	236	220	
Gender			0.217
Female	127 (53.8)	131 (59.5)	
Age (yr)			0.057
18-29	70 (29.7)	60 (27.3)	
30-49	107 (45.3)	122 (55.5)	
> 50	59 (25.0)	38 (17.3)	
Race			0.945
White	167 (70.8)	155 (70.5)	
Education			0.485
Illiterate	4 (1.7)	5 (2.3)	
Elementary School	161 (68.2)	150 (68.5)	
High School	61 (25.8)	60 (27.4)	
University Education	10 (4.2)	4 (1.8)	
City of origin			0.000
Porto Alegre	189 (80.1)	127 (57.7)	
Metropolitan region	36 (15.3)	73 (33.2)	
Countryside	11 (4.7)	20 (9.1)	
Hospital admission	160 (67.8)	161 (73.2)	0.208
Initial CD4+ T-cell count			0.075
0-200 cells/mm^3	91 (38.6)	71 (32.4)	
201-500 cells/mm^3	84 (35.6)	70 (32.0)	
> 501 cells/mm^3	61 (25.8)	78 (35.6)	
Final CD4+ T-cell count			0.518
0-200 cells/mm^3	17 (7.6)	20 (9.6)	
201-500 cells/mm^3	76 (33.8)	61 (29.3)	
> 501 cells/mm^3	132 (58.7)	127 (61.1)	
Number of CD4+ T-cell /HIV-VL samples			0.316
1-3	53 (22.6)	55 (25.2)	
4-6	103 (43.8)	104 (47.7)	
7 or more	79 (33.6)	59 (27.1)	
Number of ART schemes			0.215
1	164 (69.5)	142 (64.5)	
2	44 (18.6)	56 (25.5)	
> 3	24 (10.2)	21 (9.5)	
Time of ART initiation			0.001
Up to 1 month	117 (50.4)	103 (47.0)	
1-6 months	94 (40.5)	67 (30.6)	
6-12 months	12 (5.2)	24 (11.0)	
After 1 year	9 (3.9)	25 (11.4)	

P < 0.05 considered significant. ART: Antiretroviral therapy; HIV-VL: Human immunodeficiency virus-viral load; HNSC: Nossa Senhora da Conceição Hospital.

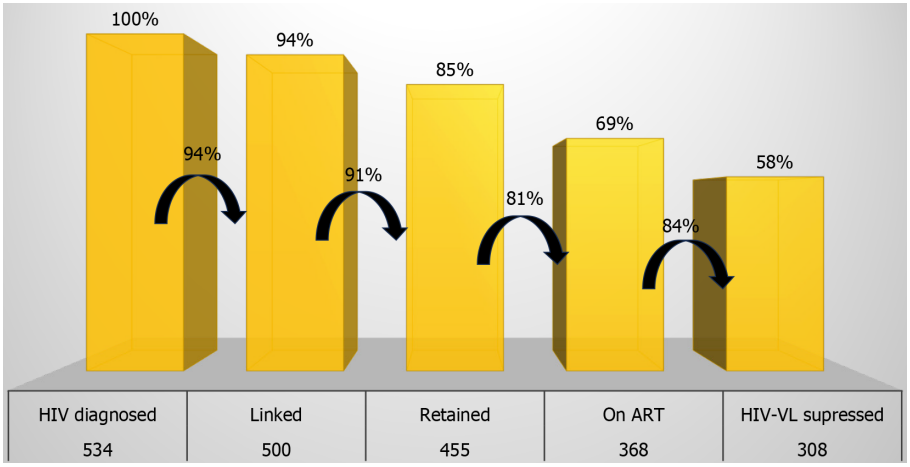

Figure 1 Human immunodeficiency virus cascade of patients diagnosed in the Nossa Senhora da Conceição Hospital in southern Brazil between 2015 and 2016, illustrated in five steps: Human immunodeficiency virus diagnosed, linked, retained, adherence to antiretroviral therapy (on antiretroviral therapy) and viral load (human immunodeficiency virus-viral load) suppression. HIV-VL: Human immunodeficiency virus-viral load; ART: Antiretroviral therapy.

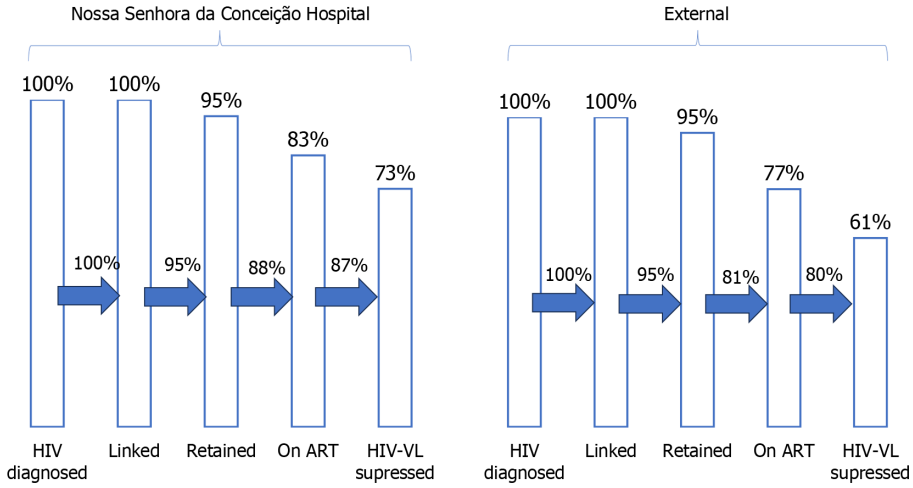

Figure 2 Human immunodeficiency virus cascades of patients followed up after hospital discharge at the Nossa Senhora da Conceição Hospital *vs* followed up at other sites (external), illustrated in five steps: Human immunodeficiency virus diagnosed, linked, retained, adherence to antiretroviral therapy (on antiretroviral therapy) and viral load (human immunodeficiency virus-viral load) suppression. HIV-VL: Human immunodeficiency virus-viral load; ART: Antiretroviral therapy.

CD4 + T-cell count of < 200 cells/mm³ in 2016[15]. In Brazil, since 2015, only 27% of people diagnosed with HIV presented with a CD4 + T-cell count < 200 cells/mm³, with a median first count of 387 cells/mm³ in 2018. A late diagnosis was related to being a man, aged > 40 years, being black, and with a low educational level (corresponding to both national and the current study's data)[16].

The analysis of the linked and retained patients considered the collection of one and two samples of CD4 + T-cell counts/HIV-VL tests, respectively, and these pillars of the cascades presented the greatest disparity in the criteria among the reviewed studies[16]. The collection of these tests refers to the performance of specialized medical care and possibly to the link to health services. In this study, the percentages related to linkage and retention to care were consistent with the UNAIDS expectations, both for the overall cascade and cascades of patients followed up at the HNSC and those followed up at other sites. The short period of time between diagnosis and the first CD4 + T-cell count can be explained mainly by the hospital protocol to request a CD4 + T-cell count/HIV-VL test immediately after positive serology results.

Of the patients diagnosed with HIV infection, 15% had no appointments at any site and 7% underwent no CD4 + T-cell count/HIV-VL test. This difference can be explained by the sample collection during hospitalization. Some of the reasons

for non-linkage and non-retention to care are stigma, fear, denial, mental health problems, lack of transportation, and health system bureaucracies[17]. The stimulus for retention in care can be provided by health professionals, with more information being provided about appointments and necessary care, social assistance and transportation, and adjusted date and frequency of appointments according to the patient's availability, in addition to providing care with cordiality and efficiency in a welcoming environment[17].

Early initiation and adherence to ART are beneficial and essential for improving the immune pattern, reducing viral replication and transmission[12,13,18]. National data indicate that ART use is recommended for all people with HIV infection, regardless of the CD4 + T-cell count, provided the early use of ART[16]. In 2018, approximately 78% of the patients diagnosed with HIV infection in Brazil initiated ART within 6 months of the first CD4 + T-cell count[9]. In 2015 and 2016, 65% and 77% of the patients, respectively, diagnosed with HIV infection in Brazil initiated ART within 6 months of the first CD4 + T-cell count collection (these percentages were lower than those reported in this study)[16].

This study shows that 85% of patients receive ART at least once after an HIV diagnosis, corroborating the trend in RS, which has progressively invested in actions to combat the epidemic[15,16]. Compared with external patients, the early prescription of ART for patients followed up at the HNSC can be explained either by the prescription of ART during hospitalization or by the early return to the HNSC infectious diseases outpatient clinic. This study identified that 37.5% of patients who did not initiate ART during the study period died. Sociodemographic and socioeconomic factors, including a patient's perception of health, stigma, fear, weak social support, and difficulty in accessing health services, may be associated with patients who never initiated ART[19]. Some of the measures to reduce the barriers between diagnosis and ART initiation are simpler regimens with fewer pills, reducing the frequency of pill collection with lower costs, and spreading the idea that new medications have minimal adverse effects and that initiating medication use before feeling sick is more beneficial[19]. The single tablet regimen (TDF/3TC/EFZ) was defined by the Brazilian protocol as being preferential from 2013 to 2016. Only in 2017, the integrase inhibitor regimen (dolutegravir) became a priority for ART treatment naive patients and for regimen switch in HIV-VL suppressed patients[12].

The main gap identified in the overall cascade occurred between retention to care and adherence to ART. Similar to what was reported in national reports, being a man, being white, a higher educational level, and older age showed a tendency toward greater adherence to ART[16]. In this study, being aged 18–49 years was considered the greatest obstacle to adherence, and was established as a criterion for the group that needs greater intervention. A place of residence in the metropolitan region can increase adherence due to the increased number of places with pharmacies providing ART. New policies with greater decentralization of specialized care could improve care for patients who live in the interior.

Patients followed up at the HNSC did not reach the UNAIDS goals for ART adherence, despite reaching a value close to 90% (88%). The early initiation of ART observed in this study may have positively influenced adherence, although there is still a need to encourage maintenance of adherence and to develop new measures to improve ART distribution and access[20].

Adherence to ART is essential for achieving HIV-VL suppression, except in elite control patients[21]. The sociodemographic risk factors for persistence of detectable HIV-VL despite ART use in Brazil are the same as those cited above for non-adherence to ART[16]. In this study, being aged 18–49 years was considered the main risk factor for the non-occurrence of HIV-VL suppression, which may be related to the low perception of risk and health care by young people. Persistent viremia can be explained mainly by poor adherence or failure to institute ART, as well as the use of less potent ART drugs than the new integrase inhibitors.

Only 84% of patients who adhered to ART achieved HIV-VL suppression. Of the patients in the HNSC and outpatient subgroups, 87% and 80% of adherent patients achieved HIV-VL suppression, respectively. The better results of patients who were followed up at the HNSC can be explained by maintenance of the link and care provided by infectious diseases physicians. Appointments with specialists who prioritize guidelines, with appropriate ART prescription and regime switches and with the provision of genotyping, when necessary, enhance patient care[22]. It was not possible to assess whether the care in external places was with a specialist physician or if the patients were seen in the same place with adequate frequency, as the only information obtained was the place of collection of the medication.

Better HIV-VL suppression results at the HNSC could be achieved mainly by increasing the number of appointments, since less than half of the patients had the two annual appointments recommended by the guidelines[12]. Prioritizing more immunosuppressed patients (CD4 + T-cell count < 200 cells/mm^3) and those at higher risk of dropping out of treatment, such as younger patients, would improve ART adherence interventions to achieve HIV-VL suppression. Thus, not only HIV-VL suppression should be considered but also its impact on the daily lives of patients, as they depend on several psychosocial, sociodemographic, and socioeconomic aspects[23]. HIV-VL suppression needs to be reproduced from the perspective of the dynamics of patients' lives, analyzing not only operational factors but also their contexts for the implementation of effective measures[23]. The main limitations of this study are related to the data missing from the medical records of the HNSC and of other sites, which were important for a thorough analysis of the sociodemographic variables.

CONCLUSION

The continuous follow-up of patients with the development of the HIV cascade with local data allows for the identification of the local HIV epidemic status. In this study, the HIV cascade of the HNSC identified the sociodemographic characteristics and subsequent health care outcomes of patients diagnosed with HIV infection in the HNSC. It was also possible to stratify and to evaluate risk factors associated with cascade leakages, such as age group, for the development of strategies directed at the 90-90-90 goal. The difference in the results obtained from the place of follow-up after

diagnosis and those from the HNSC showed the importance of specialized and continued care, to obtain outcomes close to the goals set by the UNAIDS. New studies are needed for the continuous evaluation of indicators related to HIV care.

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FOOTNOTES

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Retrospective Study

Rhabdomyolysis-related acute kidney injury in patients with COVID-19

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Abstract

BACKGROUND

Viral and bacterial infections may be complicated by rhabdomyolysis, which has a spectrum of clinical presentations ranging from asymptomatic laboratory abnormalities to life-threatening conditions such as renal failure. Direct viral injury as well as inflammatory responses may cause rhabdomyolysis in the course of coronavirus disease 2019 (COVID-19). When presented with acute kidney injury (AKI), rhabdomyolysis may be related to higher morbidity and mortality.

AIM

To compare rhabdomyolysis-related AKI with other AKIs during COVID-19.

METHODS

A total of 115 patients with COVID-19 who had AKI were evaluated retrospectively. Fifteen patients had a definite diagnosis of rhabdomyolysis (*i.e.*, creatine kinase levels increased to > 5 times the upper normal range with a concomitant increase in transaminases and lactate dehydrogenase). These patients were aged 61.0 ± 19.1 years and their baseline creatinine levels were 0.87 ± 0.13 mg/dL. Patients were treated according to national COVID-19 treatment guidelines. They were compared with patients with COVID-19 who had AKI due to other reasons.

RESULTS

For patients with rhabdomyolysis, creatinine reached 2.47 ± 1.17 mg/dL during follow-up in hospital. Of these patients, 13.3% had AKI upon hospital admission, and 86.4% developed AKI during hospital follow-up. Their peak C-reactive protein reached as high as 253.2 ± 80.6 mg/L and was higher than in patients with AKI due to other reasons ($P < 0.01$). Peak ferritin and procalcitonin levels were also higher for patients with rhabdomyolysis ($P = 0.02$ and $P = 0.002$, respective-

ly). The mortality of patients with rhabdomyolysis was calculated as 73.3%, which was higher than in other patients with AKI (18.1%) (P = 0.001).

CONCLUSION

Rhabdomyolysis was present in 13.0% of the patients who had AKI during COVID-19 infection. Rhabdomyolysis-related AKI is more proinflammatory and has a more mortal clinical course.

Key Words: Rhabdomyolysis; Acute kidney injury; COVID-19; SARS-CoV-2; Creatine kinase

Core Tip: This study investigated rhabdomyolysis-related acute kidney injury (AKI) in patients with coronavirus disease 2019 (COVID-19) and compared it with COVID-19-related AKI due to other causes. Patients with rhabdomyolysis had more inflammation with higher levels of C-reactive protein, procalcitonin, and ferritin. The prognosis of rhabdomyolysis-related AKI was worse than for other forms of COVID-19-related AKI. Patients with inflammatory viral infections such as COVID-19 should be closely followed up for life-threatening conditions such as rhabdomyolysis.

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INTRODUCTION

Rhabdomyolysis is characterized by extensive muscular injury and the release of intracellular components into the systemic circulation. The severity of rhabdomyolysis may range from asymptomatic enzyme elevations to electrolyte abnormalities and even life-threatening conditions. Symptoms of rhabdomyolysis include myalgia, muscle weakness, and red/brown urine.

Trauma, strenuous exercise, hyperthermia, and toxin exposures are the main etiologies of rhabdomyolysis, but it can also be caused by infections and sepsis[1]. Among infectious agents, viruses constitute a considerable part. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can also cause rhabdomyolysis. There are different theoretical mechanisms of rhabdomyolysis in coronavirus disease 2019 (COVID-19). One theory is direct muscle injury by SARS-CoV-2. Also, metabolic disequilibrium between energy needs and production, especially in patients with hypoxemia, can cause rhabdomyolysis[2]. The real incidence and outcome of rhabdomyolysis in the course of COVID-19 are not known. However, previous studies found the incidence at around 15% to 20%. Additionally, the mortality of patients with COVID-19 with rhabdomyolysis was found to exceed 40%[3].

Independent of rhabdomyolysis, acute kidney injury (AKI) may be seen in the course of COVID-19, and it is related to poor prognosis[4]. Rhabdomyolysis may further complicate the clinical course as an additional risk factor for AKI. Rhabdomyolysis was found as the background etiology for 7% of patients who had AKI in the course of COVID-19[5].

This study aimed to compare rhabdomyolysis-related AKI with other types of AKI in COVID-19. Such analysis may provide insights for physicians to manage different viral infections that may cause AKI and rhabdomyolysis.

MATERIALS AND METHODS

Setting and the patients

The study was conducted at Cerrahpasa Medical Faculty, Istanbul, Türkiye, which serves as a tertiary healthcare center. During the pandemic, the university hospital was designated as a pandemic control hospital. The first wave of patients with COVID-19 from the pandemic was between 15 March to 15 June 2020. To study the sole relation between COVID-19 and AKI, those who had prior chronic kidney disease (CKD) were excluded from the study. Patients who had rhabdomyolysis-related in-hospital AKI were involved in the study.

Definitions

COVID-19 was diagnosed using real-time polymerase chain reaction test from combined nasal and oral swabs.

AKI was diagnosed according to the Kidney Diseases Improving Global Outcomes (KDIGO) criteria: An absolute increase in creatinine levels by 0.3 mg/dL in the last 48 h or a 50% increase in creatinine levels in the last 7 d. AKI was also categorized according to the KDIGO criteria: A 1.5–1.9 times increase was classed as stage I, a 2.0–2.9 times increase was considered as stage II, and > 3.0 times or increase to > 4.0 mg/dL was accepted as stage III. Baseline creatinine was defined as the lowest creatinine level in the last 6 mo, excluding the last 7 d. This was detected *via* a nationwide electronic

health record system. Rhabdomyolysis was diagnosed in patients who had creatine kinase (CK) levels that were higher than five times the upper normal level with concomitant increases in transaminases and lactate dehydrogenase (LDH).

Patient follow-up

Patients who were diagnosed as having COVID-19 and had AKI were admitted to the designated COVID-19 wards in the hospital. They received all supportive care according to the guidelines released and regularly updated by the Turkish Ministry of Health COVID-19 Scientific Steering Committee. Hemogram, C-reactive protein (CRP), procalcitonin, pro-brain natriuretic peptide (proBNP), urea, creatinine, electrolyte levels, and oxygen saturation were checked daily. When needed, patients were admitted to intensive care units, receiving all necessary medical care. The endpoint for patient follow-up was either hospital discharge or death.

Statistical analysis

The normality of the distribution of continuous data was assessed using the Kolmogorov–Smirnov test. Continuous data with normal distribution are presented as mean ± SD and those with non-normal distribution are presented as median and interquartile range. Between-group analysis of continuous data was performed using the independent samples Students' t test or Mann–Whitney U test depending on the normality of the distribution. Categorical variables are presented as percentages and compared using the χ^2 test. IBM SPSS version 22.0 was used for statistical analysis. A two-sided $P < 0.05$ was accepted as statistically significant.

RESULTS

Among 115 patients who had AKI in this first wave of the pandemic, 15 had concomitant rhabdomyolysis. Two patients had AKI on the day of hospital admission and the remaining 13 developed new-onset AKI during hospital admission. The mean day of AKI was 7.4 days ± 3.1 d for these 13 patients.

In the comparison of patients with rhabdomyolysis-related AKI and other patients with AKI, age, baseline creatinine levels, and sex distribution were similar. In addition, the rates of both hypertension and diabetes between these two groups were also similar (Table 1).

The complete blood count and hemoglobin levels of patients were comparable (Table 2). The mean arterial pressure of both groups was also similar. Peak creatinine levels of patients with rhabdomyolysis were higher. In addition, patients with AKI and rhabdomyolysis had lower lymphocyte counts and higher CRP levels. Procalcitonin and peak procalcitonin levels were also higher for patients with both AKI and rhabdomyolysis. Although on-admission ferritin levels were similar for both groups, patients with rhabdomyolysis had higher peak ferritin levels indicating a greater inflammatory response.

Peak creatinine levels in the follow-up were significantly higher for patients with rhabdomyolysis. In other words, AKI was more severe for patients with rhabdomyolysis, with 80% of them having stage II or III AKI. In contrast, AKI without rhabdomyolysis was milder (84.4% of these patients had stage I AKI) (Table 3). Patients with AKI and rhabdomyolysis had significantly higher proBNP levels, higher intensive care unit admissions, and higher mortality than patients with AKI due to other causes. Mortality of rhabdomyolysis-related AKI was 73.3%, whereas it was 18.0% for other patients with AKI. However, the rate of hyperkalemia was not different for both groups.

DISCUSSION

While AKI may develop due to a variety of reasons during COVID-19[6], rhabdomyolysis is one of the specific risk factors. In patients with rhabdomyolysis, non-protein heme pigment is mainly responsible for the occurrence of AKI[7]. Although some drugs may cause rhabdomyolysis, it was not attributed to any medication in our patient cohort. In the pathophysiology of rhabdomyolysis-related AKI, vasoconstriction, tubular injury, and/or tubular obstruction can be responsible factors. Avoiding volume depletion is the main strategy to prevent AKI in rhabdomyolysis. Also, prescribing relevant fluid treatments decreases intratubular cast formation. However, fluid administration during COVID-19 was advised to be restricted, with a fear that it may result in lung edema[8].

Previously, our group and other researchers have shown that, when complicated with AKI, COVID-19 has a worse prognosis[9,10]. However, the prognosis of different etiologies for AKI was not compared in the vast majority of studies. This is mainly because of the difficulty in finding the exact etiology of AKI in the course of COVID-19. Because of the respiratory nature of the disease, acute tubular necrosis due to hypoxemia has been accepted as the main factor of AKI in COVID-19. Hypercoagulopathy and the inflammatory nature of the disease may result in intrarenal injuries including microangiopathies. The exact etiology is seldom defined because performing a kidney biopsy is not possible for most patients with unstable clinical conditions. However, rhabdomyolysis can generally be diagnosed with clinical signs, symptoms, and laboratory findings. We identified 15 patients with rhabdomyolysis among our patients with COVID-19-related AKI.

AKI was more severe in patients with rhabdomyolysis. This was apparent with higher mean creatinine levels and more patients having stage II and III AKI (33.3% and 46.7%, respectively). In contrast, the majority of AKIs in the other group were stage I (84.4%). Rhabdomyolysis-related AKI generally occurs concurrently with hyperkalemia, which may be another reason for the higher mortality. We also found that mortality was significantly higher for patients with

Table 1 Baseline characteristics of acute kidney injury patients with rhabdomyolysis and other acute kidney injury, *n* (%)

	Rhabdomyolysis (*n* = 15)	Other AKI (*n* = 100)	*P* value
Age, yr	61.00 ± 19.10	62.60 ± 13.50	0.70
Baseline creatinine (mg/dL)	0.86 ± 0.13	0.92 ± 0.15	0.25
Gender (%, male)	73.3	75.0	0.86
Hypertension	6 (40.0)	28 (28.0)	0.34
Diabetes	5 (33.3)	21 (21.0)	0.30

Data are given as mean ± SD when normally distributed. χ^2 test is used for categorical variables and Mann-Whitney *U* test is used for non-normally distributed data. AKI: Acute kidney injury.

Table 2 Comparison of rhabdomyolysis related acute kidney injury and other acute kidney injury

	Rhabdomyolysis (*n* = 15)	Other AKI (*n* = 100)	*P* value
Peak creatinine (mg/dL)	2.47 ± 1.17	1.62 ± 0.58	0.000
Mean arterial pressure (mmHg)	89.75 ± 8.63	88.63 ± 14.38	0.790
Hemoglobin (g/dL)	12.59 ± 1.83	12.26 ± 1.83	0.560
Lymphocytes (µL)	941.60 ± 311.70	1362.30 ± 676.10	0.010
Nadir lymphocytes (µL)	533.30 ± 280.60	957.90 ± 618.80	0.020
CRP (mg/L)	111.12 ± 69.91	77.45 ± 78.41	0.140
Peak CRP (mg/dL)	253.24 ± 80.67	156.95 ± 111.20	0.000
Procalcitonin (ng/mL)	0.28 [0.12-0.75]	0.10 [0.06-0.28]	0.018
Peak procalcitonin (ng/mL)	1.61 [0.40-10.60]	0.16 [0.08-1.03]	0.002
Creatine kinase (U/L)	260.00 [123.50-1057.20]	94.50 [58.0-203.70]	0.005
Peak CK (U/L)	1929.30 ± 1282.20	238.10 ± 237.0	0.000
	Median: 1514.0	Median: 126.50	
Ferritin (ng/mL)	669.0 [195.90-1403.50]	366.00 [192.50-884.0]	0.360
Peak ferritin (ng/mL)	1320.10 ± 590.0	967.10 ± 1038.70	0.020
	Median: 1267.50	Median: 657.0	
LDH (U/L)	469.40 ± 162.70	408.00 ± 350.0	0.550
Peak LDH (U/L)	964.90 ± 409.60	654.70 ± 61.80	0.030
Albumin (g/dL)	3.15 ± 0.49	3.44 ± 0.49	0.057
D-dimer (µg FEU/mL)	1.62 ± 1.34	1.93 ± 3.17	0.730
Peak d-dimer (µg FEU/mL)	23.40 ± 22.50	7.90 ± 11.07	0.030
Uric acid (mg/dL)	4.85 ± 2.18	5.81 ± 2.18	0.160
HCO_3^- (mmol/L)	21.51 ± 8.71	24.98 ± 4.53	0.220

Data are given as mean ± SD when normally distributed and as median (interquartile range) when non-normally distributed. Student's *t*-test is used for normal distributed data and Mann-Whitney *U* test is used for non-normally distributed data. AKI: Acute kidney injury; CK: Creatine kinase; CRP: C-reactive protein; LDH: Lactate dehdyrogenase.

rhabdomyolysis; however, the rate of hyperkalemia was similar for both groups. Thus, the higher mortality of rhabdomyolysis could not be attributed to hyperkalemia. Accordingly, there must be other factors that increase mortality in patients with COVID-19 with rhabdomyolysis.

Similar to prior reports, rhabdomyolysis in our patients with AKI either started late or worsened during follow-up[11]. Thus, CK, urea, and creatinine levels, as well as electrolytes should be closely monitored in viral infections with inflammatory characteristics. We performed daily laboratory checks because patients could rapidly deteriorate. We found much higher peak ferritin and D-dimer levels in patients with both AKI and rhabdomyolysis. This may point to increased

Table 3 Acute kidney injury stages and outcome of rhabdomyolysis patients and others, *n* (%)

	Rhabdomyolysis (*n* = 15)	Other AKI (*n* = 100)	*P* value
AKI stage I, II, III (%)	20.0; 33.3; 46.7	85.0; 10.0; 5.0	0.000
ICU admission	12 (80.0)	27 (27.0)	0.002
Mortality	11 (73.3)	18 (18.0)	0.001
Pro-BNP (pg/mL)	7460 [1451.0-34624.0]	583 [99.7-4203.0]	0.003
Hyperkalemia	8 (53.3)	39 (39.0)	0.270

χ^2 test is used for categorical variables and Mann–Whitney *U* test is used for non-normally distributed data. Data are given as median (interquartile range) when non-normally distributed. AKI: Acute kidney injury; ICU: Intensive care unit; BNP: Brain natriuretic peptide.

coagulation and inflammation in cases of COVID-19 with rhabdomyolysis. Higher hypercoagulopathy and systemic inflammation may be drivers of additional muscular injury.

We found higher proBNP levels in patients with AKI with rhabdomyolysis. N-terminal pro-BNP may be elevated in cardiac stress. It may also be elevated due to increased pulmonary tension in hypoxemia. Higher proBNP is independently related to a worse prognosis for COVID-19[12]. As seen in our patients, AKI may result in higher proBNP levels as a result of volume overload. Rhabdomyolysis causes more severe AKI[13] and thus results in higher proBNP levels.

Procalcitonin levels were also higher in patients with both AKI and rhabdomyolysis. Previous reports have underlined procalcitonin as a prognostic biomarker for severe COVID-19[14]. Procalcitonin is released in the inflammatory state as a response to proinflammatory cytokines. Higher peak CRP, LDH, and ferritin levels in rhabdomyolysis may show a worse inflammatory state in patients with rhabdomyolysis compared with other AKIs. Our findings show the utility of procalcitonin in the follow-up of patients with severe COVID-19.

This study had some limitations. First, the sample size was small. This is because of the exclusion of patients who already had CKD and applying strict clinical criteria to diagnose rhabdomyolysis. Nevertheless, such an approach ensured the exact accuracy of diagnosis. Secondly, kidney biopsies could not be performed due to the disease severity and scarcity of such resources during the first wave of the pandemic. Lastly, this study focused on the first wave of the pandemic.

CONCLUSION

Rhabdomyolysis may complicate the clinical course of COVID-19 and it is one of the major etiologies for AKI. When compared with AKI due to other causes, patients with rhabdomyolysis had higher inflammatory and hypercoagulopathy markers. The prognosis of rhabdomyolysis-related AKI is worse than for other AKIs.

FOOTNOTES

Author contributions: Murt A designed and conceptualized the study; Murt A and Altiparmak MR performed data acquisition, analysis, and interpretation; Murt A drafted the first version of the manuscript; and both authors commented on the consecutive versions of the manuscript and approved the final version.

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ORIGINAL ARTICLE

Retrospective Study

Ambispective epidemiological observational study of varicella-zoster virus infection: An 18 year-single-center Bulgarian experience

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Abstract

BACKGROUND

Varicella (chickenpox) and herpes zoster (shingles) are outcomes of varicella-zoster virus (VZV) infection, and understanding their incidence trends is vital for public health planning.

AIM

To conduct an ambispective epidemiological study by analyzing the main epidemiological characteristics of VZV infection during an 18 year-period (2000-2018).

METHODS

We used descriptive and epidemiological methods to characterize chickenpox in Bulgaria, the city of Plovdiv and the region for a period of 18 years (2000-2018).

RESULTS

The average incidence of varicella-zoster infection for the period 2000–2018 in the Plovdiv region was estimated at 449.58‰. The highest relative share of the infection was assessed in the month of January at 13.6%, and the lowest in the months of August and September at 2.9% (both months). The age group most affected by the infection was 1-4 years, followed by 5-9 years. This corresponds to the so-called "pro-epidemic population" - a phenomenon typical for airborne infections, confirming their mass impact on the perpetuation of VZV infection.

CONCLUSION

Our findings reveal significant insights into VZV epidemiology, including age-specific incidence rates, clinical manifestations, and vaccination impact. This comprehensive analysis contributes to the broader understanding of VZV infec-

tion dynamics and may inform evidence-based preventive measures.

Key Words: Varicella-zoster virus; Epidemiology; Incidence trends; Chickenpox; Shingles; Public health; Pro-epidemic population; Age-specific incidence; Clinical manifestations; Vaccination impact

Core Tip: This ambispective epidemiological study shows an 18-year exploration of Varicella-zoster virus (VZV) infection dynamics in Bulgaria's Plovdiv region. With varicella (chickenpox) and herpes zoster (shingles) as VZV outcomes, our findings expose a noteworthy average incidence of 449.58 per 100000 from 2000 to 2018. Notably, January peaked at 13.6%, while August and September hospitalizations were the lowest at 2.9%. The age groups most impacted, 1-4 and 5-9 years, align with the 'population pro-epidemic' concept. These outcomes demonstrated crucial insights into VZV epidemiology, guiding evidence-based preventive measures and contributing significantly to public health planning.

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INTRODUCTION

Varicella is an acute, highly contagious infectious disease caused by the varicella-zoster virus (VZV), with an acute onset, usually elevated temperature, a moderately impaired general condition and a cyclically developing pseudopolymorphic rash accompanied by itching (*i.e.*, macules, papules, vesicles, crusts)[1-3].

Years later, VZV may reactivate and cause various neurologic conditions, such as herpes zoster, postherpetic neuralgia, vasculopathy, myelopathy, retinal necrosis, cerebellitis, and zoster sine herpete. This reactivation is linked to a decline in cell-mediated immunity, typically seen in elderly and immunocompromised people. Usually, herpes zoster is a late reactivated infection characterized by a painful, dermatome-unilaterally located herpetiform rash[4,5]. It is generally associated with a reactivation of VZV after experiencing chickenpox in childhood.

In the absence of a vaccine against varicella, the number of patients each year is equal to the cohort of newborns, with 52%-78% of cases being children under 6 years of age and 89%-95% aged less than 12[3]. In different regions of the world, varicella occurs with a clear seasonality: morbidity of VZV is highest in winter and early spring[2,6].

The VZV is widespread in Europe, and in most countries the infection usually occurs between the ages of 2 and 10 years. However, in some countries, antibodies are detected at an earlier age than in other European countries. Most newborns are seropositive for VZV due to naturally acquired passive immunity from the mother during pregnancy[7].

Chickenpox is an anthroponosis. The only source of infection is contact with a person infected with chickenpox or herpes zoster[8]. The varicella virus enters the epithelial cells of the mucous membrane of the upper respiratory tract and the conjunctiva by an airborne mechanism. Therefore, entry is by the mucous membranes of the upper respiratory tract and the conjunctivae[9].

The epidemiological observational study of VZV infection holds significant importance in public health and clinical practice. Understanding the dynamics of VZV infection, including age-specific incidence rates, clinical manifestations, and vaccination impact, provides crucial insights into disease prevention and control strategies. By elucidating the epidemiological trends and risk factors associated with VZV infection, healthcare professionals and policymakers can implement targeted interventions such as vaccination campaigns and public health policies to mitigate the burden of VZV-related diseases. Therefore, conducting comprehensive epidemiological studies on VZV infection is paramount for enhancing our understanding of the disease's impact and improving preventive measures.

We aimed to retrospectively analyze the main epidemiological characteristics of VZV infection from 2000 to 2018. Specifically, we estimated the age-specific incidence rates of VZV infection, examined the clinical manifestations and complications associated with VZV, investigated the impact of VZV vaccination programs on disease prevalence, and identified any temporal trends or shifts in VZV epidemiology over the 18-year study period. By comprehensively analyzing these epidemiological aspects, we aim to contribute valuable insights to inform public health strategies, guide clinical management practices, and reduce the burden of VZV-related diseases within our population.

MATERIALS AND METHODS

Characterization of chickenpox in Bulgaria, the city of Plovdiv, and the region for 18 years (2000-2018) was carried out.

We extended our investigation to all-day kindergartens "Detstvo moe" and "Raya" (2018-2019) and the Clinic for Infectious Diseases at "St. George UMBAL" (935 patients were studied during the period 2008-2018). Of these patients, 336

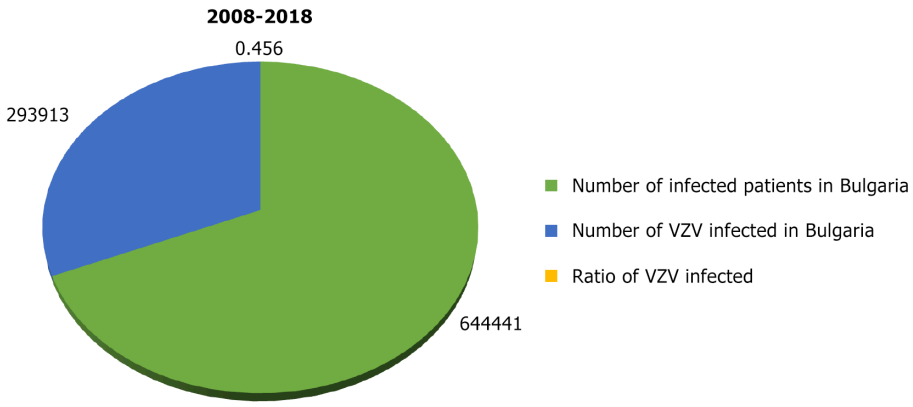

Figure 1 Relative share of patients with chickenpox from the total number of infectious patients for 2008-2018 in Bulgaria. VZV: Varicella-zoster virus.

were studied prospectively from 2012 to 2018. Additionally, the Regional dispensary for skin-venereal diseases in Plovdiv (2006-2009) was included in our analysis. We also evaluated the following: patients with established varicella zoster infection – 1104 people; patients with chickenpox in children's institutions - 107 children; hospitalized patients diagnosed with chickenpox - 935 patients; and patients hospitalized for herpes zoster - 165 patients.

We used a descriptive method to assess the real epidemic situation during the specified period and an epidemiological retrospective analysis to search for dependencies and cause-and-effect relationships and characterize chickenpox in Bulgaria, the city of Plovdiv and the region.

Statistical analysis

The obtained data were entered and processed with the statistical package IBM SPSS Statistics 25.0. MedCalc Version 19.6.3., as well as Office Excel 2021 were also used. $P < 0.05$ was accepted as a level of significance at which the null hypothesis is rejected.

RESULTS

Incidence of chickenpox in Bulgaria

According to data from the National Institute of Health and Welfare (National Center for Infectious and Parasitic Diseases)[10], for the 11 years from 2008 to 2018 in Bulgaria, 293913 people had chickenpox. The average annual morbidity was 365.52‰. During the same period, there were 644441 people in the country with infectious diseases (excluding influenza and acute respiratory infections). The relative share of chickenpox cases was 45.6% (Figure 1).

The highest relative share of chickenpox patients was observed in 2013 at 56.72% and the lowest in 2010 at 28.65%, during which period a measles epidemic also occurred. Figure 2 shows the relative share of patients with chickenpox (out of the total number of infectious patients) during 2008-2018. It was estimated below 40% in 2008 and 2010 alone (39.18% and 28.65%, respectively). In the remaining 8 years, the relative share varied from 44.97% to 56.72%.

In Figure 3A, the distribution of VZV infection cases by year is shown, and in Figure 3B, the incidence of chickenpox in Bulgaria is shown. The highest rate was in 2013 - 528.98‰ and the lowest in 2010 - 260.77‰.

Incidence of chickenpox in Plovdiv

According to the Regional Health Agency of Plovdiv data, 56502 people were registered with chickenpox from 2000 to 2018 in the Plovdiv region (Figure 4A). The average morbidity during the studied period was 449.58‰ (Figure 4B). Notably, in 2010, in the Plovdiv region, the relative share of patients with chickenpox was the lowest compared to the other years (Figure 4C). This corresponds to the lowest relative share for the whole country of Bulgaria in the same year (2010). At that time, the relative share of measles patients in Bulgaria was the highest. During 2000-2018, the incidence of chickenpox in the Plovdiv region was estimated as follows: The lowest in 2002 (220.21‰) and the highest in 2018 (666.20‰). Additionally, in Figure 4C the incidence of chickenpox in the Plovdiv region is compared with that in Bulgaria for 10 years (2009-2018).

Morbidity rate of chickenpox in Bulgaria

For Bulgaria, the trend profile for chickenpox is stationary, while in Plovdiv, it is slightly progressive (Figure 5). The reasons for this are diverse. According to data from the National Statistical Institute, the largest number of people moving to the country chose the Plovdiv region as their new place of residence, and only for 2021, immigrants totaled 21320 people (https://www.nsi.bg/bg).

On the other hand, the regions of Sofia, Varna and Plovdiv have the lowest coefficient of negative natural growth (from -3.5‰ to -6‰) in contrast to the areas of Vidin and Montana (from -18‰ to -22‰). In this way, the graph for Bulgaria,

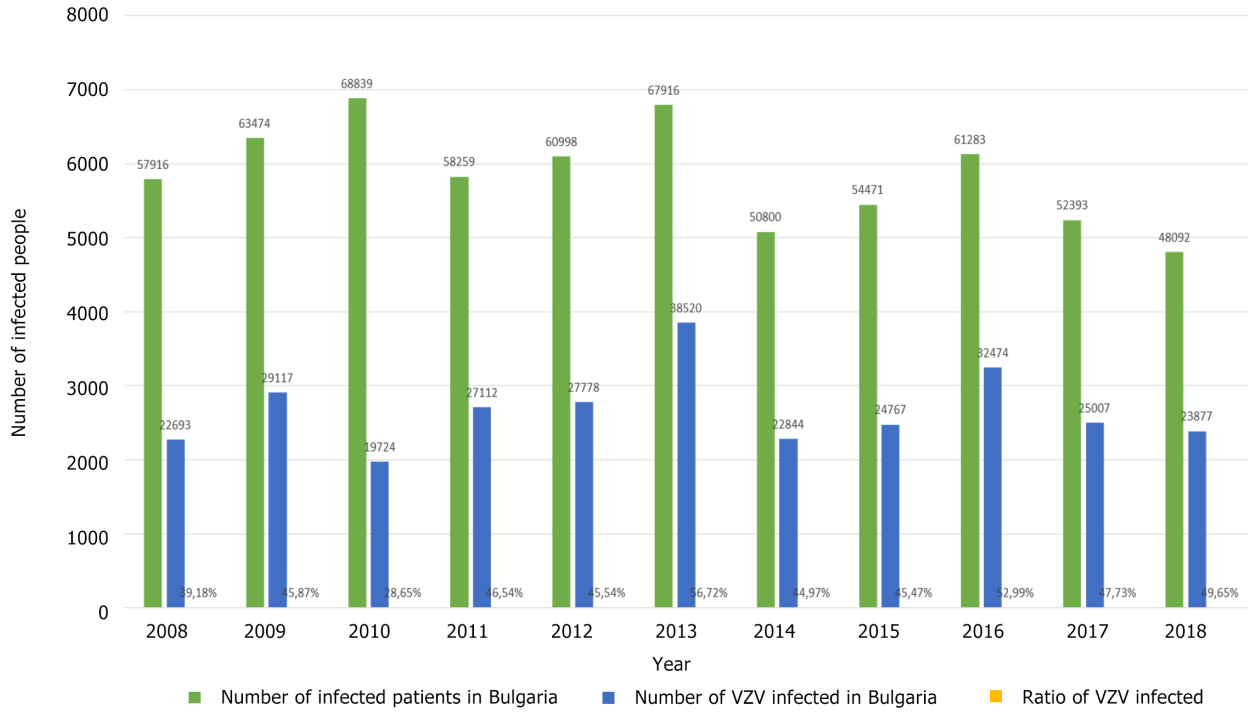

Figure 2 Relative share of patients with chickenpox from the total number of infectious patients by year in Bulgaria. VZV: Varicella-zoster virus.

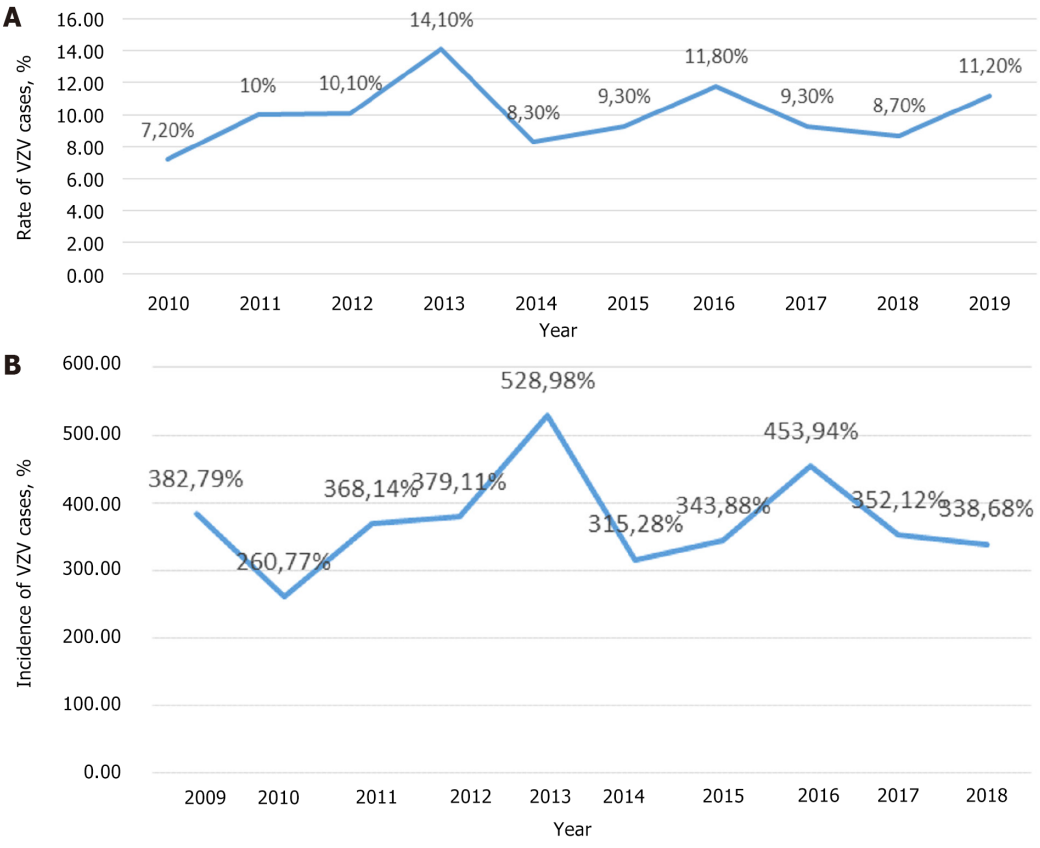

Figure 3 Distribution of varicella-zoster virus infection cases by year, and the incidence of chickenpox in Bulgaria. A: Distribution of varicella-zoster virus infection (in %) of cases by year; B: Incidence of chickenpox in Bulgaria over 10 years. VZV: Varicella-zoster virus.

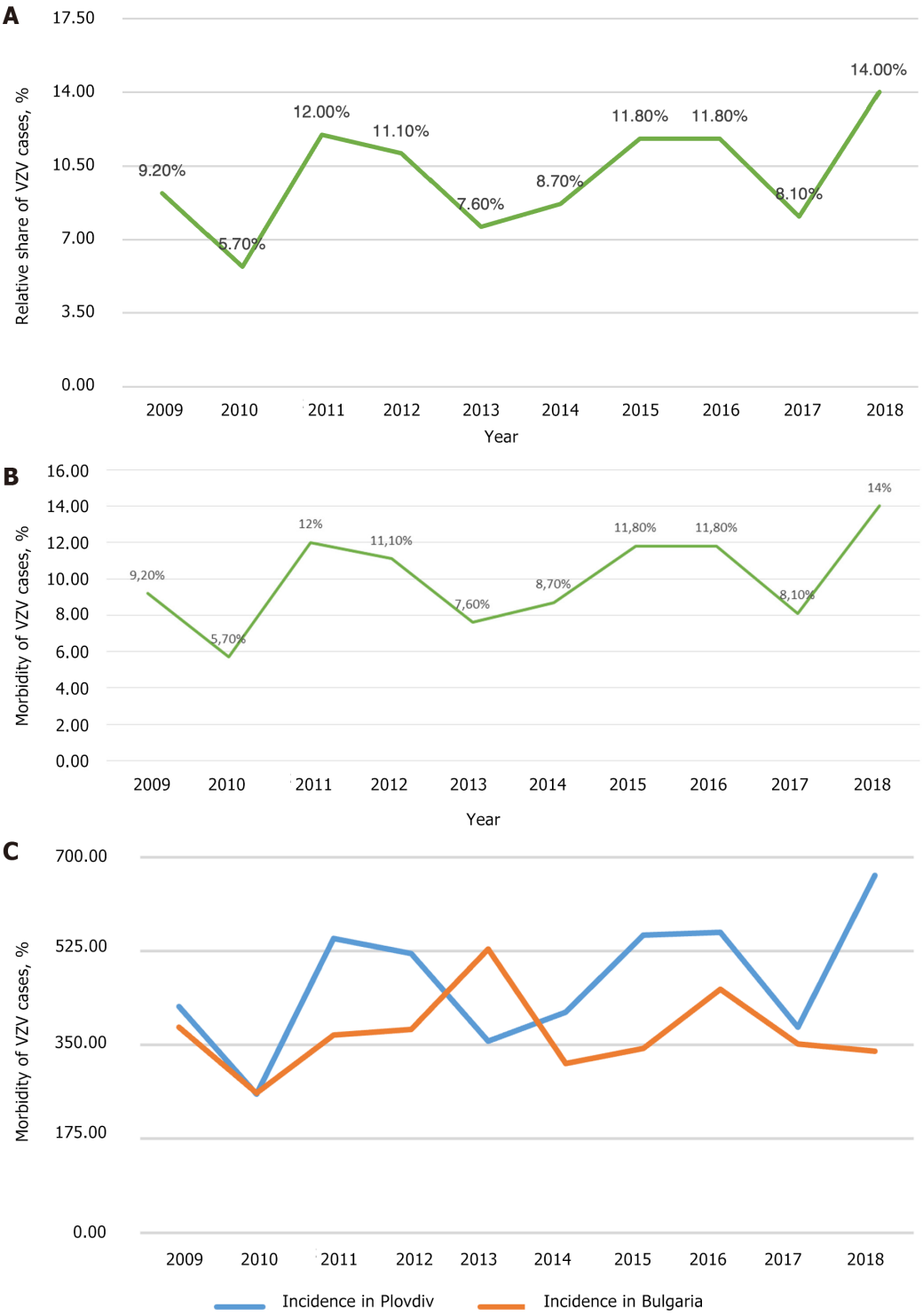

Figure 4 Incidence of chickenpox in Plovdiv. A: Distribution of the relative share of chickenpox patients in the Plovdiv region by year; B: Morbidity in Plovdiv region, 2009-2018; C: Morbidity in the region of Plovdiv and Bulgaria for the period 2009-2018. VZV: Varicella-zoster virus.

which is averaged for all regions, remains stationary. In contrast, due to the resettlement factor and the coefficient of natural growth, the graph for the Plovdiv region is slightly progressive.

It is noteworthy that in 2010, the morbidity rate was almost the same. In 2017, similar morbidity was observed again, while in 2018, the difference was considerable. Analysis of the morbidity trend in the Plovdiv region during the studied years (2000-2018) showed an upward trend (the average coefficient of increase in the number of patients was approximately 1.56) (Figure 5).

Peaks in incidence of chickenpox in Bulgaria

A statistically significant difference was demonstrated between peaks in incidence across years (P = 0.004) (data not shown visually). The peak average was 4242.00, and for the other years - 2925.19. The study period is relatively short, but

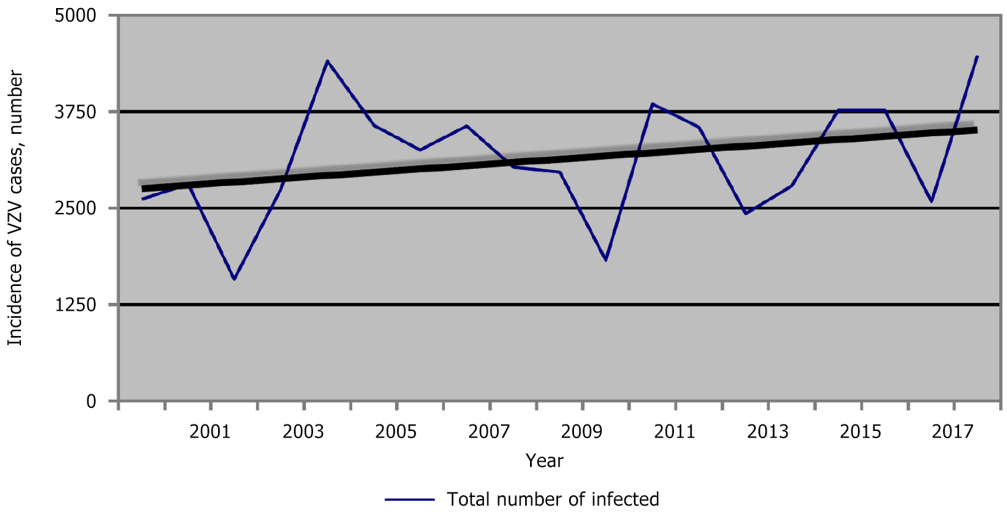

Figure 5 Trend in the incidence of chickenpox in the Plovdiv region, 2000-2018. VZV: Varicella-zoster virus.

the obtained results show that the decline in the incidence always precedes the year with the peak incidence.

We observed three peaks - in 2004, 2011, and 2018, with respective incidences of 618.21‰, 548.11‰ and 666.20‰. As expected, a substantial decline preceded each peak. We also found a cyclicity of the peaks every 7 years.

DISCUSSION

Our retrospective study showed that the average incidence of varicella-zoster infection from 2000 to 2018 in the Plovdiv region was 449.58‰. For this period, Bulgaria reported a high incidence of chickenpox, and we can speculate that this is due to the absence of mass immunoprophylaxis with the live, attenuated vaccine.

Data from the years studied showed that 2010 had the lowest relative share of chickenpox patients in the Plovdiv region. This corresponds to the data for the country at the time of the measles epidemic. From 2009 to 2018 (*i.e.*, a decade), the curve reflecting the trend of the incidence of varicella-zoster infection in the country was stationary. In the Plovdiv region, it was slightly progressive (average coefficient of increase in the number of patients was 1.56). We can explain these results with the data on internal migration. According to data from the National Statistical Institute in Bulgaria[11], most Bulgarian citizens who move to the country choose the Plovdiv region as their new place of residence. This observation mainly concerns young people with children who are potentially susceptible to the infection. These results are supported by the coefficient of negative natural growth, which for the districts of Sofia, Varna and Plovdiv is the lowest in contrast to the districts of Vidin and Montana. Besides, averaging these values explains the stationary curve of morbidity for the country and the slightly progressive trend of the curve for the Plovdiv region due to the recent population migration and the coefficient of negative natural growth.

Our observations proved peaks of high morbidity of varicella infection – higher and lower, which alternated. This is in accordance with other studies which demonstrated that demographic endemicity is determined by the continuous accumulation of a susceptible population (*i.e.*, newborns) and continuous maintenance of the infection with sporadic cases. Additionally, the cyclicity of the demographic endemicity of varicella shows how sporadic incidence turns into epidemic waves when sufficient susceptible individuals are accumulated in the population, usually every 5 years on average. However, the cycles could be small (every 2-7 years) and large (every 20-22 years)[3].

A statistically significant difference was demonstrated between peaks in incidence across years (P = 0.004) with average peaks of 4242.00‰, and in the remaining years, 2925.19‰. The summarized results of the study demonstrated further that the decline always precedes the year with the peak incidence. The observed three peaks (in 2004, 2011, and 2018, with an incidence of 618.21‰, 548.11‰ and 666.20‰, respectively) and cyclicity of 7-year intervals correspond to the studies of the investigators across different countries mentioned above.

CONCLUSION

In Bulgaria, chickenpox had a high incidence from 2000 to 2018 due to the absence of mass immunoprophylaxis with the live, attenuated vaccine. The curve reflecting the trend of morbidity in Bulgaria is of a stationary type. The epidemic process in the country and the region shows activation and decline in 7 years (known as "multi-year cyclicity").

FOOTNOTES

Author contributions: Batselova HM and Velikova TV were involved in conceptualizing the idea and writing the draft; all of the authors approved the final version of the paper prior to submission.

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Data sharing statement: No additional data are available.

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ORIGINAL ARTICLE

Observational Study

Transient elastography and diffusion-weighted magnetic resonance imaging for assessment of liver fibrosis in children with chronic hepatitis C

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Specialty type: Gastroenterology & hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

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Novelty: Grade B
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Abstract

BACKGROUND

Chronic hepatitis C (CHC) is a health burden with consequent morbidity and mortality. Liver biopsy is the gold standard for evaluating fibrosis and assessing disease severity and prognostic purposes post-treatment. Noninvasive alternatives for liver biopsy such as transient elastography (TE) and diffusion-weighted magnetic resonance imaging (DW-MRI) are critical needs.

AIM

To evaluate TE and DW-MRI as noninvasive tools for predicting liver fibrosis in children with CHC.

METHODS

This prospective cross-sectional study initially recruited 100 children with CHC virus infection. Sixty-four children completed the full set of investigations including liver stiffness measurement (LSM) using TE and measurement of apparent diffusion coefficient (ADC) of the liver and spleen using DW-MRI. Liver biopsies were evaluated for fibrosis using Ishak scoring system. LSM and liver and spleen ADC were compared in different fibrosis stages and correlation analysis was performed with histopathological findings and other laboratory parameters.

RESULTS

Most patients had moderate fibrosis (73.5%) while 26.5% had mild fibrosis. None

had severe fibrosis or cirrhosis. The majority (68.8%) had mild activity, while only 7.8% had moderate activity. Ishak scores had a significant direct correlation with LSM (P = 0.008) and were negatively correlated with both liver and spleen ADC but with no statistical significance (P = 0.086 and P = 0.145, respectively). Similarly, histopathological activity correlated significantly with LSM (P = 0.002) but not with liver or spleen ADC (P = 0.84 and 0.98 respectively). LSM and liver ADC were able to significantly discriminate F3 from lower fibrosis stages (area under the curve = 0.700 and 0.747, respectively) with a better performance of liver ADC.

CONCLUSION

TE and liver ADC were helpful in predicting significant fibrosis in children with chronic hepatitis C virus infection with a better performance of liver ADC.

Key Words: Apparent diffusion coefficient; Chronic hepatitis C; Diffusion-weighted magnetic resonance imaging; Liver fibrosis; Liver stiffness; Transient elastography

Core Tip: Although liver biopsy is not a necessity in the diagnosis of hepatitis C virus and is no longer a prerequisite for starting antiviral therapy, it remains a critical necessity to assess liver fibrosis for prognostic purposes. Noninvasive prediction of liver fibrosis is a challenging issue, especially in the pediatric population. Several studies have evaluated noninvasive serological and radiological tools for fibrosis prediction, among which are liver stiffness measurement using transient elastography (TE) and apparent diffusion coefficient using diffusion-weighted magnetic resonance imaging. The current study evaluated TE and diffusion-weighted magnetic resonance imaging compared to liver biopsy in assessing liver fibrosis.

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INTRODUCTION

Hepatic fibrosis is the end result of liver injuries in most chronic liver diseases and can lead to cirrhosis, which is complicated by the development of portal hypertension and the majority of clinical complications if a person does not seek treatment[1]. One of the most substantial problems of public health concern is hepatitis C virus (HCV) infection as clinical liver disease is extremely rare in childhood. Infection discovery is almost incidental during routine work up for other reasons or if screened for in high-risk groups[2]. Undiagnosed HCV infection in children progresses to decompensated liver disease and hepatocellular carcinoma during adulthood[3].

World Health Organization estimated that in 2022, approximately 242000 people died of hepatitis C, mostly from cirrhosis and hepatocellular carcinoma. Globally, an estimated fifty million people have chronic HCV infection, with about one million new infections occurring every year. Direct-acting antivirals (DAAs) can cure more than 95% of patients with HCV infection, but access to diagnosis and treatment is low. There is currently no effective vaccine against hepatitis C[4]. The 69th World Health Assembly approved the Global Health Sector Strategy to eliminate HCV infection by 2030, which can become a reality with the launch of DAAs[5]. The advent of DAAs has revolutionized the natural history of HCV infection with a remarkable safety profile in all stages of chronic HCV[6].

The role of liver biopsy in pediatric patients with chronic viral hepatitis was questioned due to the development of noninvasive alternatives used for the assessment of the severity of liver fibrosis. However, none of these methods has been validated in children; therefore, liver biopsy remains the gold standard for the evaluation of liver disease progression in children with chronic viral hepatitis[7]. The main indication for the liver biopsy is prognostic purposes, evaluating disease severity and monitoring response to treatment[8].

Liver biopsy is invasive, and has several limitations such as physical and mental discomfort that may lead to a significant percentage of refusals, nonnegligible morbidity (1 in 1000) and severe intraperitoneal bleeding occurs at a frequency of 1:2500 to 1:10000[9]. Attempts have been made to substitute liver biopsies with noninvasive, low-cost, reproducible methods for the evaluation of chronic hepatitis C (CHC) fibrosis[10]. Some serum markers, such as aspartate aminotransferase-to-platelet ratio index (APRI) and fibrosis-4 index (FIB-4) have been described to correlate with liver fibrosis[11].

Radiological noninvasive method based on transient elastography (TE) is a technology that is based on liver stiffness measurement (LSM) evaluated by the propagation velocity of shear waves generated in liver tissue[12]. In addition, diffusion-weighted magnetic resonance imaging (DW-MRI) is a noninvasive method that enables the measurement of the microscopic motion of water in tissue which can be recorded in the liver within an apnea period. It measures the apparent

diffusion coefficient (ADC) of water, a parameter dependent on the tissue structure. A decrease in the ADC was reported with an increase in liver fibrosis[13].

The aim of the current study was to evaluate TE and DW-MRI as noninvasive tools in evaluating liver fibrosis compared to liver biopsy in children with CHC.

MATERIALS AND METHODS

Study population

This prospective cross-sectional study initially recruited 100 children with chronic HCV infection attending the outpatient clinics of the Pediatric Hepatology, Gastroenterology, and Nutrition departments (National Liver Institute, Menoufia University, Al Minufiyah, Egypt) over a period of 3 years. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) and was approved by the institutional review board of the National Liver Institute (NLI-IRB 00003413 FWA0000227) of Menoufia University (Approval No. 0035). Written informed consent was obtained from the parents/legal guardians of the minors. All children were subjected to complete clinical examination and investigations to exclude any diseases other than chronic HCV. These included routine liver function tests, anti-HCV antibodies, quantification of HCV-RNA, other viral markers such as hepatitis B virus surface antigen, autoantibodies, protein electrophoresis, and ceruloplasmin level. Patients with other chronic liver disease associated with chronic HCV were excluded. Of the recruited children, 64 completed the study and 36 were excluded (20 children did not fulfill the complete set of investigations and 11 patients rejected liver biopsy after providing initial approval).

Serum markers of fibrosis

APRI was calculated according to the equation: APRI = [(aspartate aminotransferase (AST)/upper limit of normal)]/platelet count (10^9/L) × 100]. In addition, FIB-4 was calculated according to the equation: FIB-4 = [(age × AST)/[(platelet × alanine aminotransferase (ALT)^{½}][11].

Liver biopsy

Liver biopsies were performed under sedation and local anesthesia using the Tru-Cut needle 14 G. Formalin-fixed, paraffin-embedded specimens were examined after staining with hematoxylin and eosin, Masson's trichrome, reticulin, Perl's stains, Prussian blue and picrosirius red[14]. Significant fibrosis was defined as Ishak score of F3 or more[15].

TE

TE was performed on the right lobe of the liver using the standard M probe through the intercostal space. LSM was performed using the FibroScan apparatus (Echosens, Paris, France), which consists of a 5-MHz ultrasound transducer probe mounted on the axis of a vibrator. Mild amplitude and low frequency vibrations (50 Hz) are transmitted to the liver tissue inducing an elastic shear wave that propagates through the underlying liver tissue. The velocity of the shear wave, as measured in kilopascals (kPa), reflects tissue stiffness. Faster shear wave propagation indicates stiffer tissue[16].

DW-MRI

MRI was performed as previously described[17]. Briefly, younger children had sedation after fasting for 4-6 h. Cloral hydrate was given at a dose of 70-80 mg/kg before MRI by 30 min for younger children. Older children had MRI without sedation. MRI was performed using T1.5 T MR unit (Ingenia, Philips Best, Netherlands) using bipolar diffusion encoding gradient. Routine axial T1- and T2-weighted images were obtained. DW-MRI was done using a single shot echo-planar imaging with automatic reconstruction of ADC map.

MRI analysis

MRI interpretation was performed by one radiologist expert in MRI for 25 years (AAA). Liver and spleen ADC was automatically calculated using three consecutive slices away from vascular and biliary elements. The final ADC was represented by the mean of the three readings. Length, width and thickness of liver and spleen were also measured[17]. Normalized liver ADC was calculated as the ration of liver ADC to spleen ADC[18].

Statistical analyses

Quantitative data are expressed as the mean ± standard deviation. The statistical significance was tested according to data normality by either the *t*-test or Mann-Whitney *U* test when comparing two groups, and by either analysis of variance or Kruskal-Wallis test when comparing multiple groups. For qualitative data, significance was tested by χ^2 test or Fisher's exact test accordingly. Correlation was tested by Spearman test. Results were considered significant if $P < 0.05$. The sample size was calculated to be 60, using open epi (https://www.openepi.com/SampleSize/SSPropor.htm) with the prevalence of HCV in Egyptian children at less than 1%[19] and a confidence interval of 99.9. The cutoff values for optimal clinical performance of LSM and liver ADC were determined from the receiver-operating characteristic curve. The diagnostic performance was expressed as sensitivity and specificity percentages. The statistical review of the study was performed by a biomedical statistician. Statistical analysis was performed using SPSS, version 21 (IBM Corp., Armonk, NY, United States).

RESULTS

Study population characteristics

This study included 64 children with chronic HCV. Their age ranged between 4 years and 18 years with a mean of 12.01 ± 3.95 years and 65.4% were males. More than half of the patients (52%) had family history of HCV infection. Nearly 25% of them had hepatomegaly and/or splenomegaly. None was icteric. Most patients (82.8%) had Ishak fibrosis score of F1 or F2 while only 17.2% had F3. Other basic characteristics were as show in Table 1.

Elevated vs normal transaminases according to the different fibrosis stages

Despite different fibrosis stages, more than half of the patients had normal transaminases while the others had elevated transaminases (Figure 1). Of those with F1, F2 and F3, normal AST was found in 7 (63.6%), 20 (55.5%), and 8 (72.7%) respectively while the remaining patients had elevated AST. Similarly, normal ALT was found in most patients as indicated in Figure 1.

Serological and radiological parameters in different fibrosis stages

LSM and liver ADC were the only parameters with significant difference among different fibrosis stages (P = 0.034 and P = 0.039 respectively), while spleen ADC, APRI, and FIB-4 were nonsignificant (Table 2). Comparing LSM and liver ADC between individual fibrosis stages showed that both LSM and liver ADC were nonsignificant when comparing F1 *vs* F2. On the other hand, LSM was significantly higher in F3 when compared to F 2 (P = 0.035) and liver ADC was significantly lower in F3 when compared to F2 (P = 0.017). In addition, normalized liver ADC showed significant difference between F1 and F2, and between F2 and F3 (Table 2).

Correlation of fibrosis and activity with the studied parameters

There was a significant correlation between LSM and both of fibrosis stage (P = 0.008) and activity grade (P = 0.002), while there was no significant correlation with any of the other studied serological parameters, and liver and spleen ADC (Table 3).

Performance of LSM and ADC in discriminating significant fibrosis

LSM and liver ADC were able to discriminate significantly F3 from lower fibrosis stages with AUC of 0.700 and 0.747 respectively with a better performance of liver ADC. Sensitivity was equal for both parameters (63.3%) but with higher specificity of liver ADC (87.3%) compared to LSM (75.5%) (Figure 2).

DISCUSSION

The current study evaluated TE and DW-MRI as noninvasive tools in evaluating liver fibrosis compared to liver biopsy in children with CHC. Applying such a comparison in children needs to be addressed as it is very crucial in pediatric age group to find a less invasive alternative to detect and follow-up fibrosis[20]. In addition, guidelines on this topic are an unmet need in hepatology[21].

We found that APRI and FIB-4 did not significantly differ with different fibrosis stages or correlated with fibrosis. Contrary to our results, Güzelbulut *et al*[11] reported that APRI and FIB-4 were accurate noninvasive blood tests to predict the presence or absence of significant fibrosis and cirrhosis in adult patients with CHC. In addition, Barakat *et al* [22] studied 166 Egyptian children with CHC and found that APRI and FIB-4 could discriminate different stages of fibrosis.

In agreement with our results, other studies reported that APRI was not useful in predicting fibrosis in chronic viral hepatitis[23,24]. ElShahawy *et al*[20] reported a low specificity (57.1%) for APRI in predicting liver fibrosis in children with chronic HCV and a significant number of patients could not be correctly classified by this method[25].

The reason for the insignificance of APRI and FIB-4 in our study may be the presence of considerable number of patients (nearly 50%) with normal transaminases regardless the stage of fibrosis. Such markers are dependent on transaminases levels. In addition, previous studies included patients with higher fibrosis stages. Serum AST/ALT levels can fluctuate in those with chronic HCV and studies have demonstrated that pediatric patients can have normal AST/ALT despite histologic evidence[26]. El-Raziky *et al*[27] reported that ALT was high in 50% of patients and histopathological abnormalities were found in 75% of patients. This means that liver enzymes in chronic HCV infection do not reflect histopathological abnormalities in most cases and normal transaminases are frequently encountered in chronic HCV Egyptian patients[28].

Although TE measures the stiffness of a liver parenchymal volume 100 times bigger if compared to liver biopsy, it cannot enable segmental liver assessment and the presence of ascites or obesity represents an obstacle to its performance [29]. By contrast, DW-MRI evaluates the whole liver volume and enables segmental ADC measurements; thus, providing information about the most severely affected liver segment. In addition, it can be performed in obese patients or even in the presence of ascites[30]. ADC holds promise as a noninvasive imaging technique for assessing cirrhosis in patients with chronic viral hepatitis[31]. The performance is good when predicting severe fibrosis[32].

Our results revealed that there was no significant difference in liver ADC when comparing lower fibrosis stage (F1 *vs* F2), while the difference was significant when comparing higher fibrosis stage (F2 *vs* F3). Liver ADC did not correlate with fibrosis. Similar results was reported by Serag and Ragab[33]. The results of several studies have shown that the

Table 1 Clinical, laboratory, and histopathological characteristics

Parameter	Value
Hepatomegaly	24 (26.9)
Splenomegaly	19 (23.5)
Ascites	0.0
Aspartate transaminase (U/L)	49.72 ± 42.67
Alanine transaminase (U/L)	58.36 ± 56.40
Prothrombin time (second)	13.01 ± 1.16
Hemoglobin (g/dL)	12.42 ± 1.50
White blood cells (× 10^3/mm^3)	7.04 ± 2.46
Platelets (× 10^3/mm^3)	267.96 ± 102.50
PCR (U/mL	827319.64 ± 2415665.23
Fibrosis stage	
F1	17 (26.5)
F2	36 (56.3)
F3	11 (17.2)
Activity grade	
Absent: A0	1 (16.0)
Minimal: A1-A3	14 (21.9)
Mild: A4-A8	44 (68.8)
Moderate: A9-A12	5 (7.8)
Severe: A12-A18	0 (0)

Data are *n* (%) or mean ± standard deviation. PCR: Polymerase chain reaction.

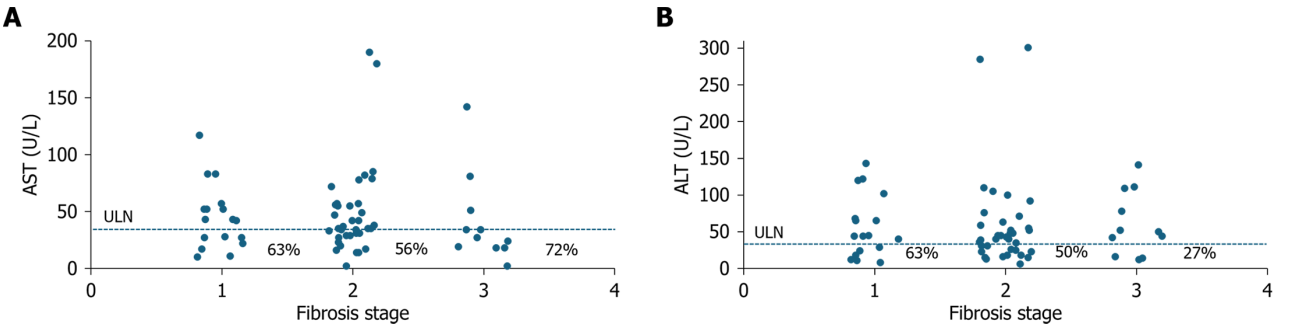

Figure 1 Normal *vs* elevated transaminases according to different fibrosis stages. A: Aspartate transaminase (AST); B: Alanine transaminase (ALT). The dashed line represents the upper limit of normal (ULN).

ADC values of cirrhotic patients are lower than those of noncirrhotic patients or of healthy volunteers[34,35], but the usefulness of the ADC in evaluating the intermediate fibrosis stages remains questionable.

Ozkurt *et al*[36] detected decreased ADC values in patients with hepatic fibrosis compared to patients with no clinical or biochemical findings of liver disease and there was a trend towards decrease in hepatic ADC values with an increasing degree of fibrosis. Taouli *et al*[37] assessed seven control subjects and 23 patients with hepatitis related liver disease. Although there was a significant difference in the ADC of the F0 and F1 groups compared with the ADC of the F2-F4 groups, there was much overlap in the ADC values of individual patients in each group. Boulanger *et al*[38] could not find a difference between the ADC values in 18 HCV patients with fibrosis due to chronic HCV and 10 healthy controls. Sandrasegaran *et al*[34] showed a significant difference in the ADC values of nonfibrotic (F0) and cirrhotic (F4) patients. However, it could not be used to reliably distinguish the intermediate stages of fibrosis.

Table 2 Liver stiffness, and liver and spleen apparent diffusion coefficient in different fibrosis stages

Noninvasive parameter	F1, *n* = 17	F2, *n* = 36	F3, *n* = 11	[a]*P* value
LSM (kPa)	4.04 ± 1.41	4.98 ± 1.63	7.20 ± 5.21	0.034
	[b]*P* = 0.096	[c]*P* = 0.035		
Liver ADC (mm^2/s)	1.12 ± 0.24	1.15 ± 0.23	0.95 ± 0.22	0.039
	[b]*P* = 0.0787	[c]*P* = 0.017		
Spleen ADC (mm^2/s)	1.12 ± 0.23	1.01 ± 0.15	0.99 ± 0.26	0.271
Normalized liver ADC	1.01 ± 0.22	1.16 ± 0.23	0.99 ± 0.25	0.036
	[b]*P* = 0.033	[c]*P* = 0.016		
APRI score	0.44 ± 0.42	0.48 ± 0.29	0.38 ± 0.37	0.332
FIB-4 score	0.34 ± 0.30	0.34 ± 0.15	0.22 ± 0.16	0.083

[a]*P*: Significance among the three groups by Kruskal-Wallis test.
[b]*P*: F1 *vs* F2.
[c]*P*: F2 *vs* F3.
ADC: Apparent diffusion coefficient; APRI: Aspartate aminotransferase-to-platelet ratio; FIB-4: Fibrosis-4 index; LSM: Liver stiffness measurement.

Table 3 Correlation of fibrosis and activity with different studied parameters

Parameters	Fibrosis		Activity	
	R value	*P* value	*R* value	*P* value
Age in yr	0.068	0.594	0.159	0.208
Aspartate transaminase (U/L)	-0.044	0.751	0.192	0.160
Alanine transaminase (U/L)	0.092	0.501	0.242	0.073
Prothrombin time (second)	-0.066	0.637	-0.184	0.188
Hemoglobin (g/dL)	0.041	0.764	-0.050	0.718
White blood cells as × $10^3/mm^3$	0.680	0.620	0.015	0.913
Platelets as × $10^3/mm^3$	0.047	0.731	-0.032	0.815
LSM (kPa)	0.338	0.008	0.385	0.002
Liver ADC (mm^2/s)	-0.299	0.086	-0.281	0.840
Spleen ADC (mm^2/s)	-0.212	0.145	-0.019	0.897
Normalized liver ADC	-0.064	0.653	-0.148	0.300
APRI score	-0.003	0.984	-0.088	0.549
FIB-4 score	-0.128	0.376	0.148	0.305

ADC: Apparent diffusion coefficient; APRI: Aspartate aminotransferase-to-platelet ratio; FIB-4: Fibrosis-4 index; LSM: Liver stiffness measurement.

Multiple studies have shown that normalized ADC, using the spleen as reference organ, improves the diagnostic performance in assessing liver fibrosis than using liver ADC alone. The spleen may be an ideal reference organ because it maintains a relatively stable ADC even in the setting of liver disease[39]. Our results showed that normalized liver ADC added no further significance over liver ADC.

The current study showed that LSM values had a significant correlation with liver fibrosis in the biopsy (P = 0.008), as well as with the activity grade (P = 0.002). There was no significant difference when comparing F1 *vs* F2, while the significance detected when comparing F2 *vs* F3 (P = 0.035).

Several studies have demonstrated the significant performance of LSM in predicting fibrosis[40,41]. Others have demonstrated discordance of LSM values with fibrosis stage and recommend that patients with a high LSM need proper attention for cirrhosis, even if liver biopsy does not reveal cirrhosis[42]. The usefulness of LSM appears not only in cross-sectional evaluation of fibrosis but also in longitudinal fibrosis follow up. Alswat *et al*[43] reported that the clearance of HCV with DAAs is associated with significant improvement in fibrosis as assessed by LSM, which supports the concept of post-treatment fibrosis regression.

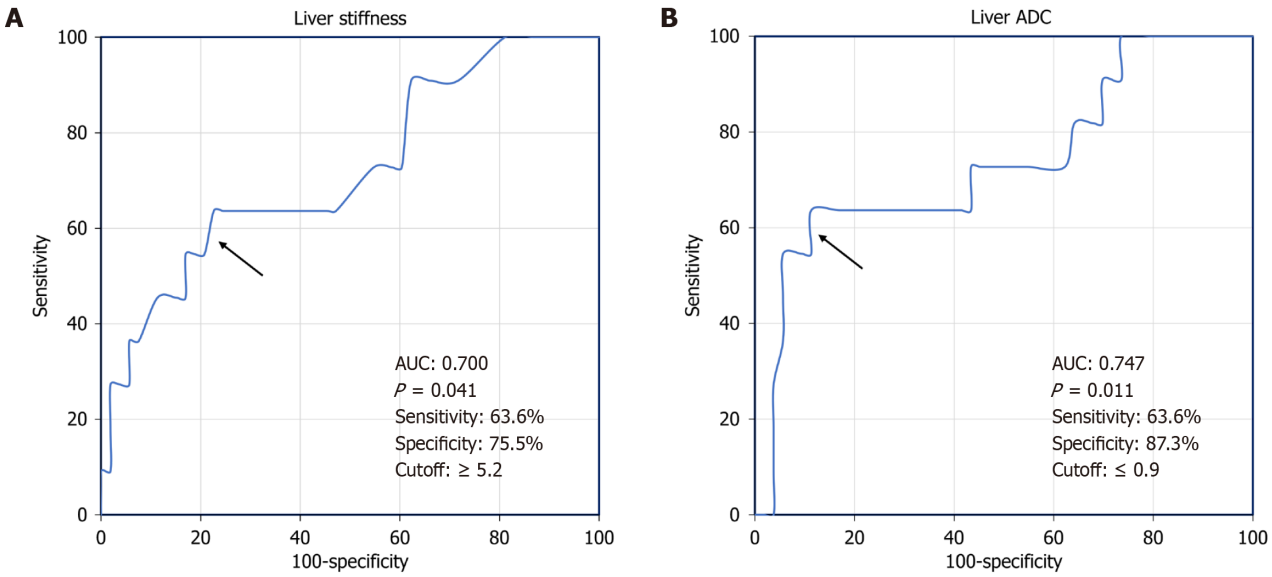

Figure 2 Diagnostic performance of noninvasive parameters of fibrosis in discriminating significant fibrosis (≥ F3). A: Liver stiffness measurement; B: Liver apparent diffusion coefficient (ADC). AUC: Area under the curve.

The limitations in our study were the limited number of patients due to dropouts, the lack of follow-up of LSM and liver ADC after the antiviral therapy, and the absence of higher stages of fibrosis.

CONCLUSION

Our study demonstrated that LSM and liver ADC were able to discriminate significant fibrosis (F3) compared to lower fibrosis stages (F1 and F2) with a better performance of liver ADC. These parameters may be of help in evaluating disease severity and their use for monitoring fibrosis post-treatment is worthy. A future multicenter study including a larger population is strongly recommended.

FOOTNOTES

Author contributions: El-Guindi MA, Sira MM, and Sobhy GA were involved in the study concept and design; El-Guindi MA, Allam AA, Sobhy GA, Salem ME, Abd-Allah MA, and Sira MM were involved in the recruitment of patients, clinical evaluation, follow-up, and contributed to data acquisition; Sira MM performed the statistical analysis and designed the figures; El-Guindi MA, Sira MM, and Sobhy GA performed the data interpretation; El-Guindi MA, Sira MM, Sobhy GA, and Salem ME wrote the manuscript; Abdel-Razek AA performed the radiological assessment and revised the first drafted manuscript; Sira MM wrote the final draft; El-Guindi MA, Allam AA, Sobhy GA, Salem ME, Abd-Allah MA, and Sira MM reviewed and approved the final manuscript.

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ORIGINAL ARTICLE

Observational Study

Recurrent stroke admissions with *vs* without COVID-19 and associated in-hospital mortality: A United States nationwide analysis, 2020

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Abstract

BACKGROUND

Coronavirus disease 2019 (COVID-19) has been shown to increase the risk of stroke. However, the prevalence and risk of recurrent stroke in COVID-19 patients with prior stroke/transient ischemic attack (TIA), as well as its impact on mortality, are not established.

AIM

To evaluate the impact of COVID-19 on in-hospital mortality, length of stay, and

healthcare costs in patients with recurrent strokes.

METHODS
We identified admissions of recurrent stroke (current acute ischemic stroke admissions with at least one prior TIA or stroke) in patients with and without COVID-19 using ICD-10-CM codes using the National Inpatient Sample (2020). We analyzed the impact of COVID-19 on mortality following recurrent stroke admissions by subgroups.

RESULTS
Of 97455 admissions with recurrent stroke, 2140 (2.2%) belonged to the COVID-19-positive group. The COVID-19-positive group had a higher prevalence of diabetes and chronic kidney disease *vs* the COVID-19 negative group ($P < 0.001$). Among the subgroups, patients aged > 65 years, patients aged 45–64 years, Asians, Hispanics, whites, and blacks in the COVID-19 positive group had higher rates of all-cause mortality than the COVID-19 negative group ($P < 0.01$). Higher odds of in-hospital mortality were seen in the group aged 45-64 (OR: 8.40, 95%CI: 4.18-16.91) *vs* the group aged > 65 (OR: 7.04, 95%CI: 5.24-9.44), males (OR: 7.82, 95%CI: 5.38-11.35) compared to females (OR: 6.15, 95%CI: 4.12-9.18), and in Hispanics (OR: 15.47, 95%CI: 7.61-31.44) and Asians/Pacific Islanders (OR: 14.93, 95%CI: 7.22-30.87) compared to blacks (OR: 5.73, 95%CI: 3.08-10.68), and whites (OR: 5.54, 95%CI: 3.79-8.09).

CONCLUSION
The study highlights the increased risk of all-cause in-hospital mortality in recurrent stroke patients with COVID-19, with a more pronounced increase in middle-aged patients, males, Hispanics, or Asians.

Key Words: COVID-19; SARS-CoV-2; Recurrent stroke; Mortality; Hospitalization; Comorbidities; Acute ischemic stroke

Core Tip: This study underscores the enhanced all-cause in-hospital mortality risk among recurrent stroke patients who test positive for coronavirus disease 2019 (COVID-19). Notably, the increased mortality risk is most significant in middle-aged individuals (45-64 years), males, and ethnic minorities, including Hispanics and Asians. Data from the National Inpatient Sample in 2020 revealed that COVID-19 patients with prior stroke or transient ischemic attack exhibit higher mortality compared to non-COVID-19 counterparts, alongside a greater prevalence of comorbidities such as diabetes and chronic kidney disease. These findings emphasize the critical need for targeted management strategies in these high-risk groups.

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INTRODUCTION

Stroke poses a substantial health burden, affecting around 795000 people annually in the United States and ranking as a leading cause of long-term disability. Nearly 1 in 4 of these occur in patients with a history of stroke[1] *i.e.*, approximately 25% of all strokes are recurrent[2]. There are several preventive strategies to reduce the risk of recurrent stroke, including medication and mitigating the risk factors[3]. Despite these preventive practices, the 5-year cumulative incidence of recurrent stroke remains relatively high, ranging from 16% to 30%[4,5]. Mohan *et al*[4], reported the cumulative risk of recurrence after first event stroke to be 3.1% at 30 days, 11.1% at 1 year, 26.4% at 5 years, and 39.2% at 10 years. To compound this issue, the emergence of coronavirus disease 2019 (COVID-19) has introduced new challenges and is linked to an increased risk of acute ischemic stroke[6,7]. In our study, we investigated the influence of COVID-19 on in-hospital mortality rates among patients admitted for recurrent stroke, comparing those who tested positive for the virus to those who did not.

MATERIALS AND METHODS

Study design and setting

The data utilized in this investigation were obtained from the National Inpatient Sample (NIS) (2020) database, an organization sponsored by the Agency for Healthcare Research and Quality. The NIS was provided with discharge data for over 7 million hospitalizations spanning 48 states by over 1000 hospital facilities[3]. This study focused on hospitalized individuals with recurrent stroke diagnoses.

Study population and data collection

Our study population was primarily composed of recurrent stroke hospitalizations. We divided these patients into two cohorts based on their COVID-19 test results: The COVID-19 positive (+) and COVID-19 negative (-) cohorts. A recurrent stroke was defined as any new acute ischemic stroke occurring with an onset separate from that of the index stroke or transient ischemic attack history. The primary outcome was in-hospital mortality. In addition, we conducted a subgroup analysis of in-hospital mortality by age, gender, and race. We evaluated demographics and baseline characteristics.

Statistical analysis

Descriptive statistics were utilized to analyze the demographics and comorbidities of the study population. We evaluated differences in baseline characteristics between the two cohorts using χ^2 tests for categorical variables and the Mann-Whitney *U* test for continuous variables (non-normal distribution). The in-hospital mortality was determined using multivariable logistic regression analyses adjusted for potential confounders. The results were presented as adjusted odds ratios (aORs) with 95% confidence intervals (CI).

RESULTS

We found that there were 97255 hospitalizations due to recurrent strokes in the NIS database for the year 2020. Among recurrent stroke hospitalizations, 2140 (2.2%) belonged to the COVID-19 (+) cohort, and 95115 (97.8%) belonged to the COVID-19 (-) cohort. The COVID-19 (+) cohort predominantly comprised individuals aged 65 and above, accounting for 72.9% of cases. The proportion of COVID-19 + patients was greater in males (53.5%) compared to females (46.5%). When examining ethnicity, whites constituted the majority of the COVID-19 (+) cohort at 53.2%, blacks at 25.1%, Hispanics at 17.2%, and Asian/Pacific Islanders at 4.2%. Regarding socioeconomic status, those in the lowest income percentile (0–25) had the highest representation within the COVID-19 (+) cohort at 36.5%. Among healthcare facilities, urban teaching hospitals had the highest proportion of COVID-19 (+) cases at 75%, compared to urban non-teaching hospitals at 16.6% and rural hospitals at 8.4%. Moreover, patients with comorbidities such as diabetes (53.0%) and chronic kidney disease (CKD) (30.8%) were more frequently found within the COVID-19 (+) cohort compared to the COVID-19 (-) cohort (Table 1).

Patients in the COVID-19 (+) cohort showed poorer outcomes, with 49.7% requiring disposition to facilities like skilled nursing facility, intermediate care facility, *etc.*, and 21.8% requiring home health care. The median length of stay was higher (7 days) for the COVID-19 (+) cohort compared to COVID-19 (-) (3 days). The median cost of hospitalization was also higher among the COVID-19 (+) cohort ($80888) compared to patients without COVID-19 ($52662) (all *P*-values < 0.005) (Table 1). Unadjusted all-cause mortality was substantially higher among recurrent stroke patients who belonged to the COVID-19 (+) cohort compared to COVID-19 (-) (22.9% *vs* 4.0%) (Table 1).

Using multivariate regression after adjusting for all factors and covariates, our analyses showed high in-hospital mortality among recurrent stroke patients with COVID-19 (+) (aOR: 7.01, 95%CI: 5.36–9.18). Subgroup analyses revealed higher odds among patients aged 45-64 (aOR: 8.4, 95%CI: 4.18-16.91) compared to age group 65 or greater (aOR: 7.04, 95%CI: 5.24-9.44); among males (aOR: 7.82, 95%CI: 5.38-11.35) compared to females (aOR: 6.15, 95%CI: 4.12-9.18). Although patients of all races exhibited significant adjusted odds of in-hospital mortality, Hispanics (aOR: 15.47, 95%CI: 7.61-31.44) and Asian Pacific Islanders (aOR: 14.93, 95%CI: 7.22-30.87) had almost three times more odds than Blacks (aOR: 5.73, 95%CI: 3.08-10.68), and White population (aOR: 5.54, 95%CI: 3.79-8.09) (All *P* value < 0.005) (Table 2).

DISCUSSION

Hospitalizations for recurrent strokes in patients who also tested positive for COVID-19 were associated with higher mortality rates longer stays in the hospital and increased total healthcare costs. Within the COVID-19 (+) cohort, it was observed that men, individuals in middle age, as well as Hispanic and Asian patients, faced a greater risk of in-hospital mortality.

Our findings align with other studies indicating a significant increase in risk-associated deaths from heart disease and stroke among COVID-19 patients, particularly among ethnic and racial minorities[8]. The heightened mortality risk is intricately linked to the interplay between inflammation and endothelial dysfunction induced by COVID-19. Severe cases trigger a cytokine storm, leading to endothelial injury and thrombotic complications. The study by Lee *et al*[9]. further elucidates how COVID-19 antibody-mediated cytotoxicity against endothelial cells initiates vascular complications, exacerbating damage in patients with recurrent stroke. The severe stroke presentations in COVID-19 (+) patients[10] can be attributed to COVID-19-induced inflammation and immune dysregulation. Additionally, decreased emergency department visits and delayed hospital admissions due to the pandemic contributed to poor outcomes[11].

While our study revealed that most patients admitted with recurrent acute ischemic stroke and COVID-19 were in the older age group (> 60 years), the adjusted odds for in-hospital mortality were higher across all age groups. Interestingly, the middle-aged group exhibited higher odds compared to other age groups. These results are consistent with findings in other studies that suggested older adults were better protected due to fewer contacts with exposure. For instance, Malmgren *et al*[12] in Washington State reported a decline in COVID-19 among older individuals and an increase in younger patients, speculating that public warnings targeted those aged 60 and older. In contrast, younger adults had more social interactions. This finding also aligns with studies that hypothesized older adults were better protected[13].

Table 1 Baseline characteristics, comorbidities and outcomes of recurrent/subsequent stroke admissions with *vs* without coronavirus disease 2019, 2020

Variables		Total recurrent stroke (AIS with prior stroke/TIA) (*n* = 97255)		*P* value
		No COVID-19 (*n* = 95115), %	COVID-19 (*n* = 2140), %	
Age in years at admission	Median (IQR)	72 (62-82)	73 (63-82)	0.001
	18-44	3.6	3.7	
	45-64	27.0	23.4	
	≥ 65	69.4	72.9	
Sex	Male	51.0	53.5	0.023
	Female	49.0	46.5	
Race	White	68.9	53.2	< 0.001
	Black	19.3	25.1	
	Hispanic	8.6	17.2	
	Asian/Pacific Islander	2.8	4.2	
	Native American	0.4		
Median household income national quartile for patient ZIP code	0-25th	31.3	36.5	< 0.001
	26-50th	27.1	30.1	
	51-75th	23.1	18.0	
	76-100th	18.4	15.4	
Payer type	Medicare	72.8	74.6	0.118
	Medicaid	10.2	9.0	
	Private	17.0	16.4	
Hospital location & teaching status	Rural	6.7	8.4	0.001
	Urban Nonteaching	18.6	16.6	
	Urban Teaching	74.7	75.0	
Hospital region	Northeast	14.5	16.8	< 0.001
	Midwest	21.1	19.2	
	South	45.2	47.9	
	West	19.2	16.1	
Comorbidities				
Hypertension		89.5	85.7	< 0.001
Diabetes		43.2	53.0	< 0.001
Hyperlipidemia		66.8	61.4	< 0.001
Obesity		14.9	16.1	0.124
Peripheral vascular disease		11.7	8.6	< 0.001
Tobacco use disorder		28.9	28.3	0.535
Prior MI		10.3	5.8	< 0.001
Prior VTE		5.6	6.3	0.162
Cancer		5.0	3.7	0.009
Chronic kidney disease		22.7	30.8	< 0.001
Alcohol abuse		4.3	2.8	0.001

Drug abuse		3.1	2.1	0.007
Depression		11.8	9.6	0.001
Chronic pulmonary disease		17.3	15.0	0.004
Hypothyroidism		15.2	14.0	0.133
Other thyroid disorders		2.3	2.1	0.502
Valvular disease		2.7	1.6	0.002
Autoimmune conditions		3.2	2.8	0.283
Outcomes				
All-cause in-hospital mortality		4.0	22.9	< 0.001
Disposition of patient	Routine	36.6	25.2	< 0.001
	Transfer to short-term hospital	2.8	3.4	
	Transfer other: SNF, ICF, *etc.*	38.6	49.7	
	Home health care	21.9	21.8	
Length of stay (days)	Median (IQR)	3 (2-6)	7 (3-13)	< 0.001
Hospital charges (USD)	Median	$52662	$80888	< 0.001

P < 0.05 is noted to be statistically significant. MI: Myocardial infarction; VTE: Venous thromboembolism; SNF: Skilled nursing facility; ICF: Intermediate care facility; IQR: Inter-quartile range; TIA: Transient ischemic attack; COVID-19: Coronavirus disease 2019.

Table 2 Multivariable logistic regression assessing impact of coronavirus disease 2019 on mortality following recurrent stroke admissions

In-hospital mortality	COVID-19	Adjusted OR	95%CI		*P* value
			Lower Limit	Upper limit	
Overall mortality	Yes *vs* No	7.01	5.36	9.18	< 0.001
In-hospital mortality by individual subgroup					
Ages 45-64	Yes *vs* No	8.4	4.18	16.91	< 0.001
Ages 65 and above	Yes *vs* No	7.04	5.24	9.44	< 0.001
Male	Yes *vs* No	7.82	5.38	11.35	< 0.001
Female	Yes *vs* No	6.15	4.12	9.18	< 0.001
Whites	Yes *vs* No	5.54	3.79	8.09	< 0.001
Blacks	Yes *vs* No	5.73	3.08	10.68	< 0.001
Hispanics	Yes *vs* No	15.47	7.61	31.44	< 0.001
Asian/Pacific Islanders	Yes *vs* No	14.93	7.22	30.87	< 0.001

P < 0.05 indicates statistical significance. Multivariable regression models were adjusted for baseline demographics, hospital level characteristics and relevant comorbidities. COVID-19: Coronavirus disease 2019; OR: Odds ratio.

Possible explanations for poorer outcomes in middle-aged include that they may have more subclinical chronic conditions like undiagnosed hypertension and poor metabolic health, leading to worse COVID-19 outcomes[14,15], and this age group may also have a dysregulated immune response leading to increased mortality[16].

Ethnic and racial disparities were evident, with Hispanics and Asian Pacific Islanders showing alarmingly higher odds of mortality. These results align with existing literature highlighting the disproportionate impact of COVID-19 on minority racial and ethnic groups, with hospitalizations being highest among Hispanic/Latino patients[17]. A multicenter case-control study conducted in England and Scotland during the first wave of the pandemic found that Asian ethnicity is strongly linked to COVID-19-related stroke, with the proportion of cases among Asians being more than twice the controls[18]. Annual United States mortality study of 2020 COVID-19-related deaths investigating the impact of socioeconomic position showed that low socioeconomic position and Hispanic ethnicity also stand out as risk factors for COVID-19-related mortality[19,20].

Adjusted subgroup analysis also showed that males had higher in-hospital mortality compared to females. Existing literature shows that there is a more significant burden of stroke deaths among women[21]. However, our study findings showed increased mortality in men compared to women. This finding could have resulted from poorer outcomes from COVID-19 disease among men. These worse outcomes among men suffering from COVID-19 could be attributed to inherent immune differences, an increased prevalence of unhealthy behaviors such as smoking and consuming alcohol, and a higher prevalence of metabolic risk factors among men[22-24].

Our study revealed an elevated prevalence of diabetes and CKD among patients with recurrent stroke and COVID-19. This finding can be attributed to the immunosuppressed nature of these conditions[25]. Diabetic and CKD patients exhibit worsened outcomes due to COVID-19 disease, including higher hospitalization rates, severe pneumonia, acute respiratory distress syndrome, the need for dialysis, and increased mortality[26,27]. Persistent hyperglycemia in diabetes hinders the immune response due to the pro-inflammatory state induced by elevated levels of inflammatory markers such as interleukin-6 and C-reactive protein, aggravating worse clinical outcomes[28]. Concurrently, CKD patients face increased thrombotic risk due to chronic inflammation and uremia[29]. Hypertension, a significant COVID-19 risk factor [30,31], predisposes infected individuals to severe disease through endothelial dysfunction-mediated hypercoagulability [32]. Exploring the impact of the COVID-19 pandemic on the management of chronic conditions reveals a pandemic-induced decline in doctor visits, particularly affecting vulnerable populations[33]. This decline exacerbates comorbidities, raising the risk of recurrent stroke as medication adherence and healthcare continuity wane[34,35]. Our study also highlights the increased risk of recurrent stroke among obese patients, consistent with current literature[36,37]. The pandemic-driven rise in average BMI and obesity prevalence, potentially stemming from decreased physical activity and a sedentary lifestyle, adds to the multifaceted challenges posed by COVID-19[38,39].

Recurrent stroke poses a significant burden on both patients and the healthcare system, with mortality ranging from 11.6% to 25.9% for in-hospital 30-day or 4-year periods[10]. Disability-adjusted life years (DALY) analysis by Hong *et al* [40] indicates a DALY loss of 3.82 after the index stroke, with an additional 0.84 DALY lost due to recurrent stroke. The average healthcare cost per person for stroke, including inpatient care, rehabilitation, and follow-up, is estimated at USD 140048[41]. Our study reports median in-hospital costs for recurrent stroke and COVID-19 at $80888, $28226 higher than those without COVID-19. Patients in the COVID-19 (+) cohort had a longer length of stay at seven days compared to those in the COVID-19 (-) cohort (3 days).

Clinical implications

Given this significant burden, our study has many future clinical implications. Rigorous management of risk factors and comorbid conditions is necessary. Timely screening, healthy behaviors, and equitable healthcare access are pivotal in reducing the disparities found in our study. Telemedicine and tailored prevention plans are crucial for achieving continuity of care and must be further explored for less severe cases.

Limitations

Through this study, we highlighted various risk factors for poor outcomes in patients admitted due to recurrent stroke and COVID-19. However, it is imperative to acknowledge the retrospective nature of our study and its inherent limitations. The observational nature of the study limits control over all potential confounders. Firstly, there might be administrative coding errors in the NIS database, which could over or underestimate COVID-19 and recurrent strokes. Variability in documentation and coding of comorbidities and outcomes across different healthcare facilities could influence the results. Secondly, our study only focuses on data from hospitalized patients, introducing potential sampling bias, as it overlooks undiagnosed or milder cases of COVID-19 not requiring hospitalization. Lack of outpatient data and long-term COVID-19 symptoms monitoring might impact results. An additional constraint of our study, stemming from its reliance on the NIS database for 2020, is the absence of vaccination status data for the subjects early in the pandemic. This limitation is particularly critical when assessing the potential mitigating impact of COVID-19 vaccines on the severe presentation of stroke, as demonstrated by Jiang *et al*[42], who found an association between even partial vaccination and a lower risk of major adverse cardiovascular events post-COVID-19 infection. Thirdly, data on the incidence of stroke among various ethnicities could have been influenced by reporting bias, as previous studies have shown a higher risk of stroke among African Americans. Finally, the data was not subclassified based on mechanisms of previous stroke, such as large vessel atherosclerosis or cardioembolic stroke. This subclassification is crucial for determining outcomes based on underlying mechanisms, and future prospective studies need to focus on incorporating this data to improve the management of these patients. In this context, future studies should focus on these limitations, and further prospective studies should be done with vaccination status data to understand the efficacy of vaccines in preventing severe manifestations and improving vaccination rates. Also, studies need to be done to understand the long-term implications of COVID-19 disease on patients with a history of stroke or TIA.

CONCLUSION

Our study shows increased in-hospital mortality after recurrent stroke and COVID-19, especially among the age group 45–64 years, Hispanics, and males. Given these findings, it is imperative to monitor risk factors such as diabetes rigorously, consistently screen them with HbA1c tests, and maintain strict glucose control, as such patients are at significant risk for recurrent stroke. Ensuring these patients stay compliant with secondary prevention strategies such as statin and antiplatelet agent use is paramount. Ethnic disparities evident in our study might be due to the lack of access and increased disease burden in these minority populations. Possible ways to reduce disparities include implementing

evidence-based practices system-wide with quality improvement initiatives and encouraging best practices. New policies must address these disparities and reinforce existing policies to improve health equity among all ethnicities. Further prospective studies are essential to unraveling the mechanisms underlying the long-term consequences of COVID-19, particularly stroke incidence. Designing a comprehensive timeline for stroke occurrences and post-Covid-19 infection will contribute to a deeper understanding of this phenomenon. As more data on the long-term sequelae of COVID-19 evolves, the risk of stroke and other thromboembolic phenomena could be better understood.

FOOTNOTES

Author contributions: Desai R and Mellacheruvu SP designed the research study; Akella SA, Mohammed AS, Hussain M, Mohammed AA, and Desai R performed the research; Akella SA, Mohammed AS, Mellacheruvu SP, Saketha P, and Sunkara P analyzed the data and wrote the manuscript; Gummadi J, Ghantasala P, and Desai R reviewed and edited the manuscript; All authors have read and approved the final manuscript.

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SYSTEMATIC REVIEWS

Dosage and utilization of dexamethasone in the management of COVID-19: A critical review

Imran Sethi, Asim Shaikh, Musa Sethi, Hira Khalid Chohan, Sheraz Younus, Syed A Khan, Salim Surani

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Abstract

BACKGROUND

The severe respiratory manifestations observed in severe coronavirus disease 2019 (COVID-19) cases are often associated with an excessive inflammatory response. Dexamethasone, a synthetic glucocorticoid, exerts its anti-inflammatory effects by inhibiting the transcription of pro-inflammatory genes and suppressing the activity of various immune cells. This mechanism has implications for mitigating the cytokine storm observed in severe COVID-19 cases. Early on in the pandemic, the Recovery Collaborative working group showed a mortality benefit of using dexamethasone in decreasing mortality in patients with COVID-19 requiring respiratory support. However, the optimal dosage of corticosteroids remains debatable. Several studies that compare different doses of dexamethasone in COVID-19 exist, but the results are conflicting.

AIM

To review the latest evidence regarding dosage, safety, and efficacy of dexamethasone in severe COVID-19.

METHODS
We followed preferred reporting items for systematic reviews and meta-analysis guidelines. A detailed literature search was conducted across PubMed, Google Scholar, and Medline to include publications up to March 2024. Our keywords included "COVID-19" "SARS-CoV-2" "dexamethasone" "corticosteroid" "steroid" and "glucocorticoid"-along with their combinations. We employed the Cochrane Risk of Bias Tool and the Newcastle-Ottawa scale to evaluate the integrity and potential of bias in the included studies. A meta-analysis was conducted using a random-effects model, assessing pooled odds ratios and mean differences, with heterogeneity gauged by the I^2 statistic and the χ^2 tests.

RESULTS
No statistical differences were found in 28-day all-cause mortality [pooled odds ratio (OR) = 1.109, 95%CI: 0.918-1.340], 60-day all-cause mortality (OR = 0.873, 95%CI: 0.744-1.024; I^2 = 47.29%), mean length of hospital stay (mean difference = -0.08 days, 95%CI: -0.001 to 0.161) and adverse events (OR = 0.877, 95%CI: 0.707-1.087).

CONCLUSION
Differing doses of corticosteroids have no clinical implications on mortality, mean length of hospital stay, and adverse events in COVID-19 patients. Additional research is required in patients requiring invasive or non-invasive ventilation.

Key Words: COVID-19; Steroids; Corticosteroids; Steroid dosage; Critical care; Corona virus

Core Tip: The role of steroid dosing in coronavirus disease 2019 (COVID-19) patients remains a critical area of focus especially concerning the reduction of COVID-19-associated mortality and improvement of overall serious outcomes. Numerous trials have recently been published evaluating outcomes with different steroid dosage regimens. Our meta-analysis shows that higher steroid dosing does not significantly improve serious outcomes and does not result in the reduction of serious adverse events. Therefore, barring additional studies evaluating the role of higher dosages according to stratification of various demographic factors, we do not recommend escalating doses of steroids to curb mortality or hospital stay duration in critical patients.

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INTRODUCTION

The onset of the coronavirus disease 2019 (COVID-19) pandemic has ushered in an unprecedented era of global healthcare challenges. The pathophysiology of COVID-19 is characterized by an excessive inflammatory response, often referred to as a cytokine storm, leading to widespread tissue damage, particularly in the lungs. This inflammatory state, coupled with the virus's ability to induce a dysregulated immune response, led to the exploration of treatments that could mitigate these effects. Among the various therapeutic strategies explored, corticosteroids emerged early as a cornerstone in managing severe respiratory complications associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Corticosteroids, due to their potent anti-inflammatory and immunosuppressive effects, have been hypothesized to temper the cytokine storm associated with critical stages of the disease, potentially reducing morbidity and mortality among the most afflicted patients. Corticosteroid use is not without risks, including potential side effects such as hyperglycemia, secondary infections, and neuromuscular weakness.

Early in the pandemic, the Recovery Collaborative working group provided significant evidence that a fixed dose of 6 mg dexamethasone daily for up to 10 days reduces 28-day mortality among patients on respiratory support. However, this study also highlighted the lack of benefits and potential for harm in patients not requiring oxygen support[1]. The National Institutes of Health recommends corticosteroids for patients with severe disease *i.e.* hypoxemia with sats ≤ 94% on room air or requiring oxygen[2].

While steroids might temper the cytokine storm associated with COVID-19, they can cause dose-dependent lymphopenia which may lead to prolonged viral replication[3]. The optimal dosage of corticosteroids remains debatable [4]. A limited number of studies that compare different doses of dexamethasone in COVID-19 exist, but the results are conflicting. The COVID Steroid 2 trial and the Highlowdexa trial compared lower and higher doses of dexamethasone and showed a trend towards improvement with higher steroid doses; however, this was not statistically significant[5,6]. On the other hand, other trials have shown increased mortality with higher doses of dexamethasone[7-9].

This review examines the efficacy and safety profiles of high-dose *vs* low-dose corticosteroids in treating COVID-19, contributing to a more nuanced understanding of their clinical application.

MATERIALS AND METHODS

Framework and search strategy

This study was designed following the PRISMA guidelines for systematic reviews and meta-analyses[10], focusing on the nuanced dosing of dexamethasone in treating COVID-19. A detailed literature search was conducted across PubMed, Google Scholar, and Medline to include publications up to March 2024. Our keywords included "COVID-19" "SARS-CoV-2" "dexamethasone" "corticosteroid" "steroid" and "glucocorticoid"—along with their combinations. These were meticulously chosen to align with the study's core questions. We also incorporated Medical Subject Headings to ensure the comprehensiveness of the search.

Inclusion and exclusion criteria

The scope encompassed only randomized controlled trials examining dexamethasone dosing in adults (18 years and older) with COVID-19. The primary evaluation metric was all-cause mortality at day 28 and at day greater than 60, with secondary assessments on hospital stay length and adverse effects like thrombosis, myocardial infarction, arrhythmias, and secondary infections. Due to translation constraints, exclusions were applied to non-randomized trials, single-arm studies, observational studies, case reports, and non-English publications.

Study selection and data extraction

Eligibility was initially determined through title and abstract screening by two independent reviewers (Sethi M and Shaikh A), followed by detailed full-text assessments against the inclusion criteria. Any discrepancies were resolved *via* discussion or third-party adjudication. Details are shown in Prisma flow sheet Figure 1. Using a bespoke extraction form, we collated data on study specifics, participant demographics, intervention and control details, and outcomes. The most comprehensive data set was selected and cross-referenced for studies reported multiple times with related publications. Additional study data collected included country of origin, study design, disease type and severity, and specific corticosteroid dosage regimens.

Quality assessment

We employed the Cochrane Risk of Bias Tool and the Newcastle-Ottawa Scale to evaluate the integrity and potential of bias in the included studies, addressing selection, performance, and detection biases.

Data synthesis and analysis

We opted for a narrative synthesis approach to anticipate study variability. Where applicable, a meta-analysis was conducted using a random-effects model, assessing pooled odds ratios and mean differences, with heterogeneity gauged by the I^2 statistic and the χ^2 tests. All data was collected and synthesized using Microsoft Excel 365, Redman, Washington, United States. All meta-analyses were conducted using Comprehensive Meta-Analysis Version 4, Borenstein M, Hedges L, Higgins J, and Rothstein, Biostat, Englewood, New Jersey. Funnel plots were not made as publication bias findings are contingent on including ten or more studies *per* analysis, a requirement we did not meet.

RESULTS

Search outcome and selection criteria

Our initial search yielded 6845 records. Through screening based on our established inclusion criteria, we narrowed down to 9 studies[5-9,11-15] for detailed analysis.

Demographics and study details

These selected studies collectively examined 2740 individuals diagnosed with COVID-19, aged between 18 and 85 years. The patient cohort was evenly divided between treatment protocols: 564 participants were assigned to high-dose dexamethasone groups, receiving between > 6 mg *per* day, while the other half were placed in low-dose groups, each receiving a standard daily dose of < 6 mg *per* day. The study characteristics of all included studies are shown in Table 1.

Mortality outcomes

Analysis from nine studies revealed negligible distinctions in 28-day all-cause mortality between higher and lower dexamethasone dosages [pooled odds ratio (OR) = 1.109, 95%CI: 0.918-1.340; I^2 = 66.95%]. Consistency was observed in 60-day all-cause mortality, with no notable disparities between dosing approaches (OR = 0.873, 95%CI: 0.744-1.024; I^2 = 47.29%). The forest plots of 28 and 60 days mortality are shown in Figures 2 and 3.

Table 1 Study characteristics of all included studies

Ref.	Study design	Country	Disease severity	Low-dose dexamethasone regimen	High-dose dexamethasone regimen	Outcomes studied
Bouadma *et al* [12] (2022)	Randomized multicenter trial	France	Hypoxemia	6 mg daily for 10 days	20 mg on days 1-5, then 10 mg from days 6-10	Invasive mechanical vent at 28 days, mortality at 60 days
Maskin *et al*[6] (2022)	Randomized multicenter trial	Argentina	Patients with ARDS	6 mg daily for 10 days	16 mg on days 1-5, then 8 mg from days 6-10	Ventilator-free days at 28 days, mortality at 28 and 90 days, infection muscle weakness, hyperglycemia
Granholm *et al* [13] (2021)	Randomized multicenter trial	Denmark, India, Sweden, Switzerland	Oxygen at least 10 L *per* minute, NIV or IMV	6 mg daily for 10 days	12 mg daily for 10 days	Days alive without IMV, circulatory support, renal replacement at 28 days, mortality at 28 and 90 days
Rabascall *et al*[14] (2022)	Randomized multicenter trial	United States	Patients with hypoxemia sats < 94%	6 mg daily	0.2 mg/kg	Invasive mechanical vent at 28 days, mortality at 28 days
Recovery Collaborative group[7] (2023)	Randomized multicenter trial	Asia, United Kingdom, and Africa	Patients with COVID-19 with simple Oxygen	6 mg daily for 10 days	20 mg on days 1-5, then 10 mg from days 6-10	Invasive mechanical vent at 28 days, mortality at 28 days
Taboada *et al*[11] (2022)	Randomized multicenter trial	Spain	Hypoxemia requiring Oxygen	6mg daily for 10 days	20 mg on days 1-5, then 10 mg from days 6-10	Clinically worsening in 11 days. mortality at 28 days, IMV requirement, mortality at 60 days
Toroghi *et al*[8] (2022)	Randomized multicenter trial	Iran	Hypoxemia requiring Oxygen	8 mg daily for 10 days	Intermediate dose: 8 mg BID for 10 days High dose: 8 mg TID for 10 days	Need for mechanical ventilation, 60 days mortality
Wu *et al*[9] (2022)	Randomized single-center trial	United States	Hypoxemia requiring Oxygen	6 mg daily for 10 days	20 mg on days 1-5, then 10 mg from days 6-10	Clinical improvement on day 28, mortality at 28 days
Sadeghi *et al*[15] (2023)	Randomized multicenter trial	Iran	Hypoxemia requiring Oxygen	8 mg daily for 7 days	24 mg daily for 3 days, then 8 mg daily for 4 days	Mortality at 1 month

BID: Twice *per* day; IMV: Intermittent mechanical ventilation; NIV: Noninvasive ventilation; ARDS: Adult respiratory distress syndrome; COVID-19: Coronavirus disease 2019.

Hospitalization duration

Our analysis indicated a marginally reduced average hospital stay in patients administered low-dose dexamethasone compared to those on a high-dose regimen, though this reduction did not achieve statistical significance (mean difference = -0.08 days, 95%CI: -0.001 to 0.161; I^2 = 49.48%). A forest plot comparing hospital length of stay is shown in Figure 4.

Adverse events

No difference in occurrence rates of occurrence of a composite of significant adverse effects (thrombosis, infections, myocardial infarction, and arrhythmia) was observed (OR = 0.877, 95%CI: 0.707-1.087; I^2 = 14.73%). A forest plot of the incidence of adverse effects between low-dose and high-dose steroids is shown in Figure 5.

Quality assessment

The 9 studies selected were assessed to have a low risk of bias.

DISCUSSION

Our meta-analysis, adhering to PRISMA guidelines, has evaluated the comparative efficacy and safety of high-dose *vs* low-dose dexamethasone in treating COVID-19. The data synthesis from multiple studies underscores the need for significant differences in mortality rates between the two dosing strategies. Our findings advocate for the continued use of low-dose dexamethasone, aligning with current clinical guidelines and echoing the pivotal conclusions of the Recovery trial. This approach not only mitigates mortality associated with severe COVID-19 but also minimizes certain adverse events, such as hyperglycemia and secondary infections. There is strong evidence for using low-dose dexamethasone in pts with COVID-19 on simple oxygen, as a higher dose significantly increases the risk of death[7].

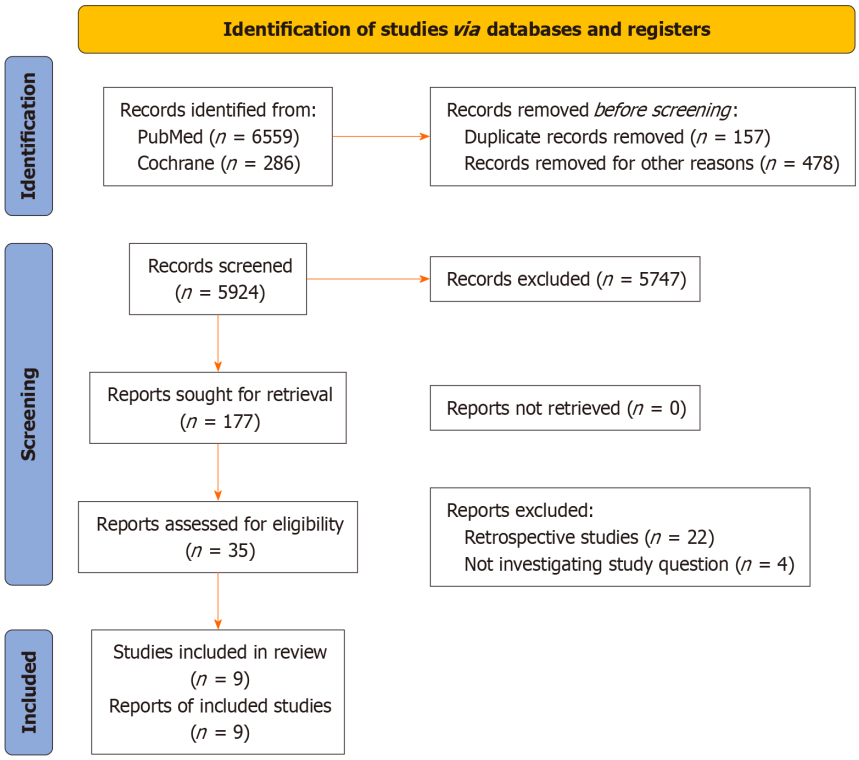

Figure 1 Study prisma flow chart.

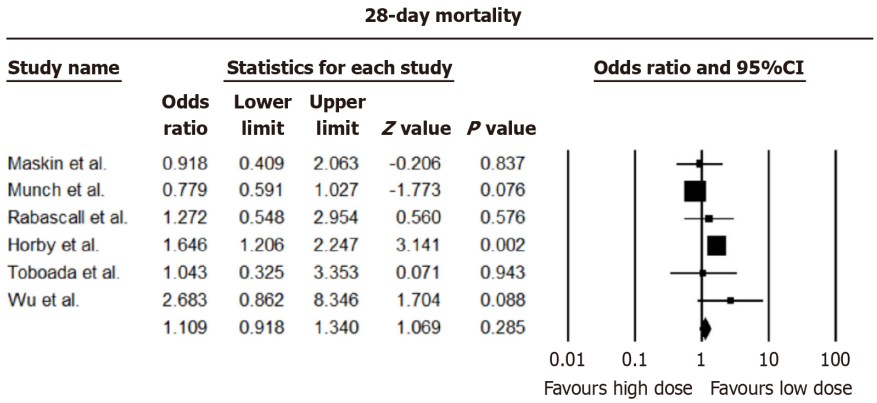

Figure 2 Pooled odds ratio for 28-day mortality rates.

However, the possibility of a benefit of high-dose corticosteroids remains. The conventional and the Bayesian analysis of the COVID Steroid 2 suggested that a dose of 12 mg might benefit patients who require noninvasive ventilation (NIV) or mechanical ventilation[5]. Also, a long-term (180) day follow-up of this trial showed that fewer patients have died in the 12 mg dexamethasone group compared to the 6 mg group (33.7% *vs* 38.6% patients). However, this finding did not reach statistical significance. This leaves open the possibility that this study needed to be underpowered. This would bolster the rationale for repeating a low *vs* high dose corticosteroid randomized controlled trial in patients with very severe respiratory impairment (NIV or invasive mechanical ventilation).

Most patients in the COVID Steroid 2 did not receive additional immunomodulators beyond steroids. In severely or critically ill patients, additional anti-inflammatory therapies like IL-6 inhibitors may be more effective at reducing mortality[16]. Currently, there are no data from steroids that evaluated the safety and efficacy of using higher doses of steroids in combination with other immunomodulators to treat hospitalized patients with COVID-19.

A single-center retrospective observational study in Spain early on in the pandemic evaluated 573 patients with severe COVID-19 treated with high pulse-dose corticosteroids (methylprednisolone ≥ 250 mg/day) *vs* standard dose (methylprednisolone 1.5 mg/kg/daily). High-dose steroids were associated with increased mortality than standard-dose (adjusted OR = 2.46, 95%CI: 1.59-3.81, $P < 0.001$) and with an increased risk of needing mechanical ventilation or death (adjusted OR = 2.35, $P = 0.001$). However, interaction analysis showed that high-dose steroids increased mortality exclusively in elderly patients[17]. This would argue for modulating *vs* completely suppressing immune response in elderly patients.

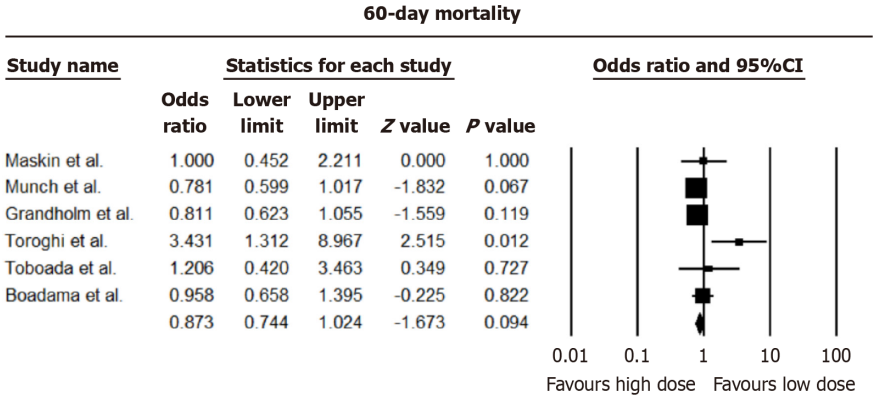

Figure 3 Pooled odds ratio for 60-day mortality rates.

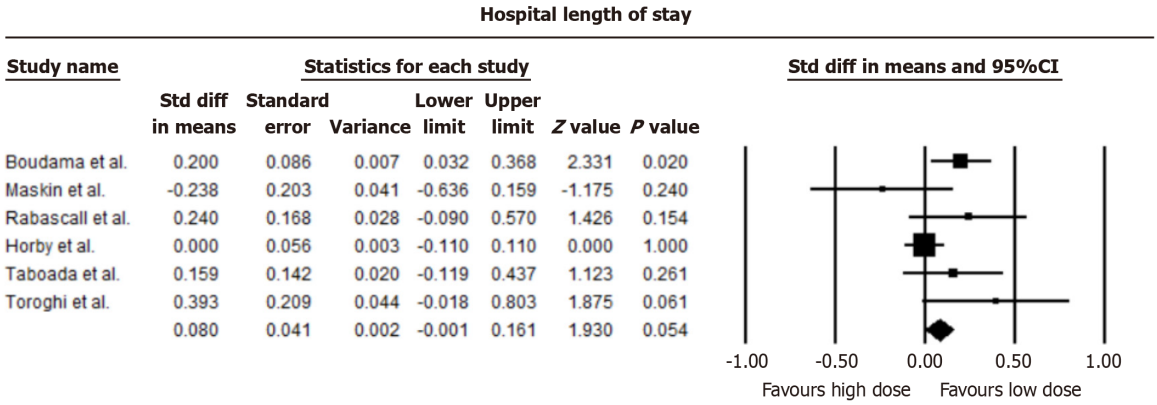

Figure 4 Comparison of average length of hospital stay.

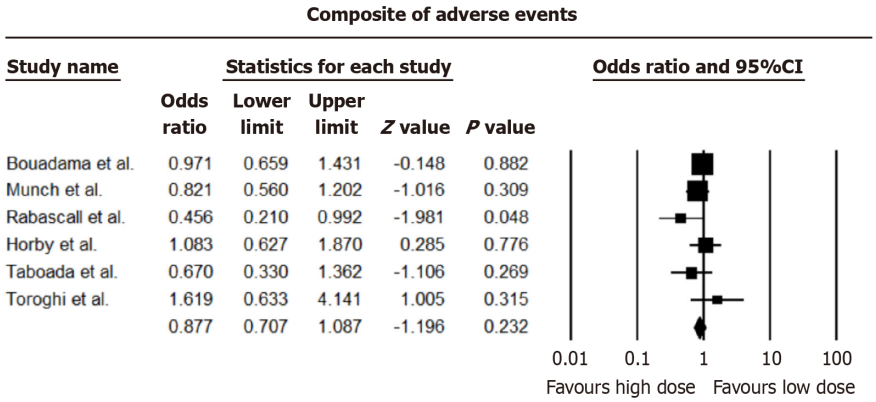

Figure 5 Incidence of adverse events in high-dose *vs* low-dose groups.

On the other hand, some small cohort studies have suggested that pulse dose steroids may indeed have a mortality benefit[18,19]. There is also some evidence that biomarker-based steroid treatment was associated with lower odds of mortality and mechanical ventilation[20,21].

Nevertheless, our study has its limitations. The inherent heterogeneity of included studies necessitates a cautious interpretation of our results. Patient demographics and disease severity levels differed between studies. In addition, these studies did not differentiate between vaccinated and unvaccinated patients.

CONCLUSION

Our comprehensive review substantiates the efficacy and safety of low-dose dexamethasone as a cornerstone treatment

for severe COVID-19. Low-dose steroids provide a balanced treatment approach that improves patient outcomes while minimizing risks. Future research should delve deeper into the stratification of patient subgroups, exploring whether certain demographics (age, gender, race, body mass index, immunity status, disease severity, or elevated biomarkers warrant deviation from standard dexamethasone dosing. Additionally, the long-term impacts of different dosing regimens on long COVID-19 remains a critical area of inquiry.

FOOTNOTES

Author contributions: Sethi I provided the conceptualization; Sethi I, Shaikh A, Sethi M, Chohan HK, and Younus S provided the literature review, drafting, and reviewing; Sethi I, Shaikh A, Sethi M agreed to the final accuracy of the work; Khan SA and Surani S provided supervision, idea generation, and critical final review of the manuscript.

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