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EDITORIAL

Impact of COVID-19 pandemic on routine childhood vaccinations

Shi-Rong Lv, Ming-Ke Wang, Xue-Lu Yu, Xin-Yue Li, Ji-Shun Yang

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Shi-Rong Lv, Ming-Ke Wang, Xue-Lu Yu, Xin-Yue Li, Department of Disease Control and Prevention, Naval Medical Center of PLA, Naval Medical University, Shanghai 200052, China

Ji-Shun Yang, Medical Care Center, Naval Medical Center of PLA, Naval Medical University, Shanghai 200052, China

Co-corresponding authors: Ming-Ke Wang and Ji-Shun Yang.

Corresponding author: Ji-Shun Yang, MD, PhD, Director, Medical Care Center, Naval Medical Center of PLA, Naval Medical University, No. 338 Huaihai West Road, Changning District, Shanghai 200052, China. jasunyang@foxmail.com

Abstract

Routine pediatric vaccination is one of the most effective public health interventions for the control of a number of fatal diseases. However, during the coronavirus disease 2019 pandemic, routine pediatric vaccination rates were severely affected by disruptions of health services and vaccine confidence issues. Governments and the United Nations have taken measures to re-establish routine pediatric vaccination, while additional efforts are needed to catch up and develop plans to ensure routine vaccination services for the future pandemics.

Key Words: Vaccines; Childhood immunizations; COVID-19; SARS-CoV-2; Pandemic

Core Tip: The impact of the coronavirus disease 2019 pandemic on vaccination coverage is critical to prevent vaccine-preventable diseases during or even after the pandemic. Exploring alternative approaches to enhance pediatric vaccination rates is imperative for the sustained improvement of global public health.

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INTRODUCTION

Vaccines are immunizing agents for the prevention of infectious diseases caused by pathogenic microorganisms and their metabolites, which are artificially attenuated, inactivated or genetically modified. Vaccines not only provide individual protection to those vaccinated, but also produce herd immunity by reducing the disease's spread among the population when reaching a certain percentage of the immunized population, thereby protecting those who are unable to be vaccinated for certain reasons[1]. Additionally, vaccines possess economic benefits, with deaths and disabilities prevented by vaccination leading to the increased economic productivity in addition to the reduced healthcare expenditures[2]. It has also been demonstrated that vaccination can slow down the rise of antimicrobial drug resistance and address global health threats by directly reducing the incidence of susceptible and drug-resistant infections or decreasing the overall incidence of disease, thereby reducing the appropriate and inappropriate use of antimicrobials[3].

Infectious diseases are one of the leading causes of death in children due to their underdeveloped immune system as susceptible to numerous infectious diseases[4]. Early-life infections may potentially result in unfavorable growth and developmental delays, exerting negative influences on long-term health and cognitive abilities[5]. In order to protect children from serious and even fatal infectious diseases, the World Health Organization (WHO) launched the Expanded Programme on Immunization in May 1974. Thereafter, vaccination lists and schedules have been established to promote child vaccine coverage and broaden the scope of vaccination.

Currently, almost all countries have routine immunization schedules in place to ensure routine childhood vaccination coverage. Routine pediatric vaccination has significantly reduced morbidity, disability, and mortality from vaccine-preventable diseases[6]. The WHO globally estimated that vaccines may prevent 2-3 million deaths annually. Moreover, due to the usage of vaccines, a wide range of infectious diseases that have caused severe health and life-threatening damage to children throughout history have been largely controlled; for instance, smallpox has been eradicated from the world, in which several regions such as the Americas, the Western Pacific, and Europe have been certified as polio-free, and the prevalence of infectious diseases (*e.g.*, measles and mumps) has been remarkably reduced[7].

However, routine pediatric vaccination coverage is affected by several factors. A recent review published in *World Journal of Virology* by Locke *et al*[8] studied and examined the influences of coronavirus disease 2019 (COVID-19) on infant, child, and adolescent vaccinations. This review revealed a decline, delays, or interruptions in the routine pediatric vaccination during the pandemic, with a reduction in some countries' pre- and post-COVID-19 pandemic eras, and emphasized the importance of governmental efforts in the maintenance and reduced delays in routine childhood vaccinations. Beyond variations in vaccination coverage attributed to differences in country, region, ethnicity, or economic status, contemporary attitudes toward vaccines play a pivotal role in influencing vaccination coverage. Diseases that were once feared and inspired a desire for vaccination are now remarkably less prevalent, and some publics are skeptical about vaccinations due to concerns arising from side effects of vaccinations[1]. In addition, regional or worldwide outbreaks may cause a reduction or interruption in vaccination coverage, including regional conflicts and pandemics[9-11]. The COVID-19 outbreak that started in December 2019 globally caused disruptions or reductions in vaccination coverage, in which routine pediatric vaccination coverage was also severely affected. In the present study, the influences of the COVID-19 pandemic on the routine pediatric vaccination and the corresponding coping and preventative strategies were discussed.

METHODOLOGY

The MEDLINE and PubMed databases were search to retrieve articles that were published in English using associations of search queries related to the concepts of COVID-19 (severe acute respiratory syndrome coronavirus 2), pandemic (epidemic, outbreak), pediatric (childhood), and vaccination (vaccine). Further references were added by hand-searching in the relevant literature, and they were included in the final manuscript based on consensus among all authors.

ROUTINE PEDIATRIC VACCINATION DURING THE COVID-19 PANDEMIC

Locke *et al*[8] retrospectively analyzed changes in routine childhood vaccination coverage during the COVID-19 pandemic and its epidemiological factors, and they found that routine vaccination coverage was severely affected by COVID-19, especially among children in the United States. Routine childhood vaccines, such as diphtheria-tetanus-pertussis (DTP) vaccine, measles, mumps and rubella (MMR) vaccine, and polio vaccine exhibited a decrease in vaccination coverage, with DTP and MMR vaccines showing a reduction of more than 7.0% in vaccination coverage compared with expectations. Kujawski *et al*[12] assessed the influences of the COVID-19 pandemic on pediatric and adolescent vaccination administration in the United States, and also revealed substantial disruptions in vaccination administration for children and adolescents during the COVID-19 pandemic in 2020 and early 2021. Other studies reported that childhood vaccination rates significantly decreased in some regions and countries, such as Alabama[13], Singapore[14], and Brazil[15] during the COVID-19 pandemic. Carias *et al*[16] projected measles vaccination coverage for the cohort of children, and found that projected vaccination coverage would drop to 80% or below after stay-at-home orders were lifted during the pandemic. Therefore, sustained catch-up immunization efforts were needed.

The COVID-19 pandemic affected immunization programs worldwide, and it led to millions of children missing out on vaccinations, contributing to a substantial rise in the count of zero-dose and incompletely vaccinated children[17]. From the commencement of the COVID-19 epidemic until 2021, there was a general decrease in global childhood vaccination

rates[18]. Childhood vaccination rates had recovered in 2022, while still had not returned to pre-epidemic rates, and the recovery level was uneven across countries[19]. In addition to the adverse effects of social determinants of health such as unemployment, poverty, and disruption of education[20], the lack of vaccine supply due to shortage of health resources and health manpower contributed to the decline in vaccination coverage during the epidemic[21]. Moreover, skepticism about the safety, efficacy, and necessity of vaccines, as well as concerns about their side effects, contributed to the decline in vaccination coverage, with the extent and magnitude of the problem increasing further during the epidemic[20]. Another study demonstrated that vaccination confidence is lost mainly among young people, which is a worrying trend that may have long-term public health consequences[22]. Furthermore, Kraaijeveld *et al*[23] pointed out that healthy children's routine vaccination against COVID-19 was ethically unjustified.

COPING AND PREVENTATIVE STRATEGIES

Interruptions in vaccination have a negative impact on children's health, and studies found that interruptions in routine vaccination could increase the risk of mortality from vaccine-preventable diseases by 10.0%. Moreover, race, age, and place of residence are all factors that influence vaccine interruptions and routine vaccination coverage. The authors recommend that governments take proactive measures to address missed vaccinations by prioritizing catch-up programs. Moreover, communities should establish a more inclusive system ensuring universal access to vaccines for all children [8]. Some countries and the United Nations had also taken steps to restore and compensate for interruptions in routine vaccination coverage[24]. The WHO, together with the United Nations Children's Fund and the Global Alliance for Vaccines and Immunization, and other organizations, have called 2023 a year of intensified action to "catch up", calling for child immunization services to be restored to pre-pandemic levels[19]. Shet *et al*[21] proposed several actions to help restore vaccination, including identifying and implementing catch-up vaccination strategies, strengthening vaccine routine information management systems, adapting health planning and establishing best practices to more effectively respond to the future epidemics.

CONCLUSION

Vaccination is an important measure to protect the population from infections and detrimental influences of vaccine-preventable diseases. Increasing vaccination coverage not only reduces the incidence of several diseases and their serious consequences and improves the humans' health, but also plays a pivotal role in the economy and in addressing global health threats. A decline in vaccination coverage for various reasons may negatively affect children's health and even threaten their future lives. Therefore, innovative actions and strategies should be taken to restore the stagnation or even decline in routine pediatric vaccination coverage during the COVID-19 pandemic and minimize their public health effects in the future.

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ORCID number: Shi-Rong Lv 0009-0000-8481-4957; Ming-Ke Wang 0000-0001-9918-0491; Xue-Lu Yu 0000-0002-8527-2093; Xin-Yue Li 0009-0003-3895-5515; Ji-Shun Yang 0000-0001-7160-706X.

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EDITORIAL

Hepatitis E virus infections

Basavraj S Nagoba, Abhijit S Rayate

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Basavraj S Nagoba, Department of Microbiology, Maharashtra Institute of Medical Sciences & Research (Medical College), Latur 413531, India

Abhijit S Rayate, Department of Surgery, Maharashtra Institute of Medical Sciences & Research (Medical College), Latur 413531, India

Corresponding author: Basavraj S Nagoba, PhD, Assistant Dean, Professor, Department of Microbiology, Maharashtra Institute of Medical Sciences & Research (Medical College), Vishwanathpuram, Ambajogai Road, Latur 413531, India. dr_bsnagoba@yahoo.com

Abstract

Hepatitis E virus (HEV) infection is now endemic worldwide. Most patients with acute infection recover uneventfully. Outbreaks and sporadic cases, particularly in high-risk individuals are emerging increasingly. The patients with risk factors like pregnancy and pre-existing chronic liver disease, present with or progress rapidly to severe disease. Immuno-suppression in post-transplant patients is an additional risk factor. Standardized FDA-approved diagnostic tests are the need of the hour. Further studies are needed to establish guideline-based treatment regimen and outbreak preparedness for HEV to decrease global morbidity, mortality, and healthcare burden. Policies for screening donors and transplant cases are required.

Key Words: Challenges; Chronic hepatitis E; Hepatitis E virus; Post-transplant hepatitis; Risk factors

Core Tip: In this editorial we have tried to throw light on various aspects of hepatitis E virus, that help in better understanding about the virus, disease process, various diagnostic approaches, treatment and prevention. We are sure that this will be helpful to plan proper surveillance and management protocols with improved outcomes.

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INTRODUCTION

The Hepatitis E virus (HEV) was considered a shy little member of the *Hepeviridae* family, playing only in some endemic nations. Over the past three decades, it has picked up the pace to emerge as an equally concerning issue as with other hepatitis viruses. Multiple outbreaks and grave outcomes of HEV in immunocompromised patients may be a whistleblowing reflection on public sanitation and surveillance measures. Insufficient knowledge of clinico-pathological features, limited resources for diagnosis, lack of systematic surveillance, and poor sanitation appear to be major reasons for poor suspicion index, late diagnosis, and inadequate estimate of disease burden due to HEV.

THE VIRUS

Five genotypes (HEV-1 to 4 and 7) are associated with human disease. HEV-1 is the most prevalent[1,2]. HEV infections in developed nations and developing nations differ in genotype, mode of transmission, outbreak characteristics, and presentations[3]. Hepatitis due to HEV-1 and 2 (less commonly 4 and 7) are prevalent in low- and mid-income nations. Genotypes 3 and 4 are common in European and other developed nations[1]. They are responsible for autochthonous cases, zoonotic-related cases, and HEV in immunocompromised patients[1,4-7].

Poor food and water sanitation and poor sewage management are primarily responsible for the feco-oral spread of HEV[1,3,8]. Zoonotic transmission may also occur due to contaminated or undercooked animal products or seafood[3,5,8,9]. Transmission through blood and blood products is a recently observed route[3,10]. The seroprevalence of IgG antibodies is variable according to the endemic regions. The presence of IgM antibodies and HEV RNA in samples from blood banks and hemodialysis units is concerning[7,11].

Labeled as travel-associated diseases, Humanitarian aid workers, immigrants and refugees, immunocompromised travelers, and travelers to low- and middle-income countries, are the new categories of vectors for spread[1,8]. We believe that medical tourism, the war and its consequences, and the relief measures during war and natural calamities, have contributed to worldwide endemicity. Person-to-person transmission has not been reported[1,5].

THE ROUTINE

HEV enters through contaminated water or food (undercooked animal products in particular), reaches the liver by the entero-portal circulation, and targets hepatocytes using intracellular machinery to replicate. In many patients, humoral and cellular immunity restricts the viral replication. The host clears out the infection, self-limiting the illness[12]. This host response is but a double-edged sword. The hepatocellular damage is mainly immune-mediated. The lymphocytosis (CD8+ and natural killer cells) and increased levels of cytokines (Interferon-gamma and interleukin-10) limit and clear out the infection at the cost of intrinsic damage to hepatocytes and resultant hepatitis[2,13,14].

Most infected people remain asymptomatic. Some patients present with acute hepatitis with the prodromal phase followed by the icteric phase. Management is usually supportive. The phase lasts over a week or so with a very low mortality rate[9,15,16]. A literature search revealed that HEV-3-related acute disease is known to progress to chronicity in immunocompromised and a small number of immunocompetent hosts[3,7,8,16,17].

THE SPECIALS

Chronic HEV and immunocompromised

In a histological study, Lenggenhager observed that HEV can have different presentations regarding clinical and histological findings and outcomes, depending on immune status and preexisting liver disease[6]. Differences due to geographical pattern and genotype have also been noted[6,14]. Patients with pre-existing liver disease are at increased risk for severe HEV infection, liver failure, and grave outcomes[5,16-18]. Despite ribavirin treatment, some of these patients may progress and need a liver transplant[5].

Chronic HEV infection predominantly occurs in immunocompromised hosts, solid organ transplant patients, HIV patients (low CD4 < 200), and chemotherapy patients. HEV-3 and less commonly HEV-4, result in this manifestation[7,9,19]. Chronic hepatitis is defined as having a carrier state in a post-transplant patient for six months or more and persistent HEV replication, three months after the acute phase[20]. Chronic HEV in post-transplant patients runs an unpredictable course and outcome[18]. Seroprevalence has been reported in both adults and pediatric recipients of liver transplants in developed nations and is increasing[19]. Chronic HEV in liver recipients may have an accelerated progression to liver cirrhosis and failure[18].

Drugs like Janus kinase inhibitors, therapies for rheumatoid or autoimmune diseases, and CD20-directed therapy for haematologic disorders, affect B-cell immunity and modulate HEV replication and subsequent hepatitis[6,10,21]. A substantial number of these patients progress to chronicity[10]. HEV hepatitis in patients with hematological malignancies needs dose adjustments of chemotherapeutic drugs and even it can delay the definitive management of underlying malignancy[10,22].

HEV and pregnancy

HEV genotype-1 is associated with fulminant hepatitis of pregnancy[1,16]. Poor host response, as evident by low counts of CD4 and CD8, high interferon, and higher viral load, contributes to higher prevalence in endemic areas. Pregnant patients are more susceptible to HEV-induced acute liver failure, increasing the odds of mortality by seven times[23]. Reported mortality in pregnant patients is 5% to 31% along with a significantly high risk for fetal loss, prematurity, and vertical transmission[2,8,23,24]. Deterioration and resultant outcome are unpredictable and may not be affected by pregnancy status[25-28]. Survivors of vertical transmission have a self-restricting infection without delayed/prolonged effects[29].

Extrahepatic manifestations

Literature review shows many extrahepatic manifestations but pathophysiology is unclear. Both altered host-immune response and direct cytotoxicity by the virus are hypothesized for these complications. Reported disorders include neurological disorders like Guillain-Barre syndrome, Bell's palsy, polyradiculopathy, neuralgic amyotrophy, acute transverse myelitis, acute meningoencephalitis, glomerulonephritis, pancreatitis, biliary disorders, hematological complications like aplastic anemia, thrombocytopenia and Monoclonal gammopathy of unknown significance. HEV-associated pancreatitis and pancreatitis in existing HEV hepatitis are poorly understood phenomena[2,5,15,30,31]. Genotypes 1 and 3 are associated with renal and neurological pathologies[1,15,16]. Immunocompetent patients are more likely to have neurological complications for unknown reasons whereas renal complications (glomerulonephritis) do not have such predilections for immune status[15].

THE CHALLENGES

Diagnosis and surveillance

The non-standardization, cost, and limited availability restrict the usage of diagnostic assays in surveillance. Serological tests are now widely available, with anti-HEV IgM antibodies common for all genotypes[7]. The Anti-HEV- IgM antibodies are detectable in 1-4 wk of infection but closely followed or overlapped by IgG. Thus, single serology cannot definitely conclude acute infection as in other viral illnesses[2,3,32]. IgG may remain serologically detectable over many years[3,32,33]. Overall specificity of various IgM tests is > 99% with sensitivity in the range of 85%-87.5% for immuno-compromised patients[32].

Immuno-compromised patients may have delayed or undetectable seroconversion. Nucleic acid amplification testing (NAAT) is useful for detecting HEV RNA from stool, serum, or liver biopsy[2,32]. Though a gold standard, NAAT has some pitfalls. RNA is detectable in the serum up to 3-4 wk after onset of illness and up to six weeks in the stool[5]. Patients with chronic HEV infection will show the presence of HEV RNA in serum/shed for a longer duration. Viral capsid antigen can be detected in blood before clinical presentation. This low-cost and easy-to-perform test may be valuable in blood screening[32]. Polymerase chain reaction and its advancements are also available[3]. Molecular assays to detect the open read frame (ORF)/conserved regions on the viral genome can contribute to better genotyping according to prevalent areas[34].

Treatment

Though no definitive approved options are available, Ribavirin is effective and widely used in the treatment of cases of HEV-associated fulminant hepatitis or chronic liver failure and other HEV complications[10,32,35,36]. Ribavirin clears the HEV virus by depleting guanosine triphosphate pools, thus inhibiting HEV-RNA replication[37]. The risk for hemolytic anemia is collateral damage, requiring monitoring.

Chronic HEV in solid-organ transplant recipients need dose adjustments of immunosuppressive agents that target T-lymphocytes. This step alone can lead to HEV clearance of patients[5,10,38]. In addition to dose reduction, treatment may include Ribavirin and Pegylated interferon-alpha[7,36,39]. Ribavirin is the safe and preferred agent to be consumed for three months or as per the response. The prophylactic role of Ribavirin has not been reported. Teratogenicity is under evaluation. Many researchers are evaluating the combinations of other direct-acting antiviral agents and ribavirin.

Being a virus, HEV requires host cell machinery for replication. Amidino-rocaglates are translation initiation inhibitors and are under research. In-vitro results of three Amidino-rocaglates (CMLD012073, CMLD012118, and CMLD012612) appear promising[40].

Standardized guidelines for the treatment of neurological and other extrahepatic complications are not available but ribavirin is effective in management and HEV clearance[2,5,35].

Prevention

Improved standards of sanitation and vigilance during traveling regarding food and water, remain the mainstay of prevention. The Chinese Center for Disease Control and Prevention has approved Hecolin vaccine after studies[3,9]. A safe and effective vaccine with long-lasting effects is still in its primary stage of development. WHO had advocated the use of Hecolin during the recent outbreak of 2022 in Sudan with the acceptability of the vaccine in about one-sixth of the concerned population[41]. Safe and effective vaccine with long-lasting effect is still in its primary stage of development. Vaccines, wherever available, should be considered in at-risk patients like pregnant ladies in endemic regions, solid organ transplant recipients, and chronic liver disease patients[3].

Artificial intelligence and HEV

With support of artificial intelligence (AI), researchers have integrated external/environmental factors and disease trends for issuing an alert/early warning about important infectious diseases like malaria, dengue, influenza, *etc.* Role of AI in hepatology (surveillance, diagnosis and treatment response prediction) is still in its primary stage with a majority of the work done with respect to Hepatitis B and associated disorders[42,43]. Fieulaine *et al*[44] had used AI tool known as AlphaFold2 for understanding the pORF1, which has a key role in viral replication. Time series prediction models like recurrent neural networks and Support Vector Machines are being used for disease and case-load prediction[45]. Peng *et al*[46] proposed an ensemble-based learning model that can correlate data on past HEV epidemics and various environmental factors, to give a better prediction analysis. Researchers are studying other models like long- and short-term memory networks, and other deep learning models to correlate environmental and meteorological data for better prediction over traditional models[47,48].

These advances can help the health care system to plan ahead of a HEV epidemic. Available literature is limited to analysis in certain provinces only. Global applicability and cost-effectiveness are the new targets for research.

CONCLUSION

Scientific knowledge and awareness about HEV among the medical fraternity and general population help to prevent a neglected approach during outbreaks and future consequences. Given its clinical significance and little-known mechanisms, we consider it is high time to act by vigilant research on HEV.

FOOTNOTES

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Country/Territory of origin: India

ORCID number: Basavraj S Nagoba 0000-0001-5625-3777; Abhijit S Rayate 0000-0002-6183-7029.

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EDITORIAL

Combined prevention and treatment measures are essential to control nosocomial infections during the COVID-19 pandemic

Jing-Wen Liu, Yue-Yue Li, Ming-Ke Wang, Ji-Shun Yang

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Jing-Wen Liu, Yue-Yue Li, Ji-Shun Yang, Medical Care Center, Naval Medical Center of PLA, Naval Medical University, Shanghai 200052, China

Jing-Wen Liu, Yue-Yue Li, School of Pharmacy, Bengbu Medical College, Bengbu 233000, Anhui Province, China

Ming-Ke Wang, Department of Disease Control and Prevention, Naval Medical Center of PLA, Naval Medical University, Shanghai 200052, China

Co-corresponding authors: Ming-Ke Wang and Ji-Shun Yang.

Corresponding author: Ming-Ke Wang, MD, PhD, Associate Chief Physician, Department of Disease Control and Prevention, Naval Medical Center of PLA, Naval Medical University, No. 338 Huaihai West Road, Changning District, Shanghai 200052, China. wmke021@163.com

Abstract

Severe acute respiratory syndrome coronavirus-2 is a highly contagious positive-sense, single-stranded RNA virus that has rapidly spread worldwide. As of December 17, 2023, 772838745 confirmed cases including 6988679 deaths have been reported globally. This virus primarily spreads through droplets, airborne transmission, and direct contact. Hospitals harbor a substantial number of confirmed coronavirus disease 2019 (COVID-19) patients and asymptomatic carriers, accompanied by high population density and a larger susceptible population. These factors serve as potential triggers for nosocomial infections, posing a threat during the COVID-19 pandemic. Nosocomial infections occur to varying degrees across different countries worldwide, emphasizing the urgent need for a practical approach to prevent and control the intra-hospital spread of COVID-19. This study primarily concentrated on a novel strategy combining preventive measures with treatment for combating COVID-19 nosocomial infections. It suggests preventive methods, such as vaccination, disinfection, and training of heathcare personnel to curb viral infections. Additionally, it explored therapeutic strategies targeting cellular inflammatory factors and certain new medications for COVID-19 patients. These methods hold promise in rapidly and effectively preventing and controlling nosocomial infections during the COVID-19 pandemic and provide a reliable reference for adopting preventive measures in the future pandemic.

Key Words: COVID-19; SARS-CoV-2; Nosocomial infection; Prevention; Treatment

Core Tip: During the coronavirus disease 2019 (COVID-19) pandemic, nosocomial infections severely challenged healthcare professionals worldwide. Mitigating intra-hospital diseases remains a crucial task for healthcare practitioners. This study concentrated on pivotal strategies for preventing and controlling COVID-19 infections within medical facilities. It advocated viral infection prevention through vaccination, disinfection, and the training of heathcare personnel. Simultaneously, it explored therapeutic strategies involving cellular inflammatory factors and certain new medications tailored for COVID-19 patients. These methods could prevent and control intra-hospital infections swiftly and effectively during the COVID-19 outbreak.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a highly contagious single-stranded RNA virus, rapidly spread worldwide, leading to an infection known as coronavirus disease 2019 (COVID-19). This viral infection manifests in various symptoms, including cough, fever, chest discomfort, and, in severe cases, respiratory distress syndrome[1]. As of December 17, 2023, 772838745 confirmed cases including 6988679 deaths have been reported globally[2]. This directly threatens human life and health and presents new challenges to the healthcare systems. Hospitals, with their dense population and mainly inadequate ventilation, provide an environment conducive to viral transmission[3], primarily through droplets, airborne particles, and direct contact[4]. Hospitals frequently harbor numerous confirmed COVID-19 cases, including asymptomatic carriers, posing a threat of intra-hospital infections during the COVID-19 pandemic. Since the viral outbreak, multiple hospitals have reported COVID-19 infections. For instance, the University Hospital of Muenster witnessed an intra-hospital attack of novel coronavirus infections in its pediatric dialysis unit, involving 48 cases[5]. Nine contacts tested positive for laboratory-confirmed COVID-19 infection, while two cases who tested positive remained asymptomatic. Eleven patients reported flu-like symptoms, while their testing result was negative. Between January 1 and February 9, 2020, 110 out of 9 684 healthcare workers at Wuhan Tongji Hospital tested positive for COVID-19[6], reflecting an infection rate of 1.1%.

Hence, there is an urgent need to develop effective measures to control intra-hospital transmission of SARS-CoV-2. The initial distribution of vaccines by the end of 2020 significantly reduced the rates of hospitalization, mortality, and infection rates, proving to be the most effective tool against the COVID-19 pandemic. While disinfection methods have played a crucial role, practical implementation is necessary. Implementing disinfection practices not only aids in the current pandemic but also serves as an efficient strategy for the future similar outbreaks.

PREVENTION MEASURES

Vaccination for infection prevention

With the continuous mutation of SARS-CoV-2 and the emergence of variants of concern, effective and safe vaccines are paramount in controlling the COVID-19 pandemic[7]. According to the data released by the World Health Organization (WHO) on March 28, 2022, 153 vaccines have been authorized for clinical trials, while 196 vaccines are undergoing preclinical trials. These COVID-19 vaccines mainly fall into several categories: inactivated vaccines, viral vector vaccines, RNA vaccines, DNA vaccines, protein subunit vaccines, and virus-like particle vaccines[8]. As of March 28, 2022, a total of 10 vaccines (including 3 Indian vaccines) comprising inactivated, viral vector, mRNA, and protein subunit vaccines have been approved by the WHO for emergency use (Table 1). Pharmaceuticals such as Pfizer-BioNTech's BNT162[9], Oxford-AstraZeneca's AZD1222, Sinovac's CoronaVac, Moderna's mRNA-1273, Johnson & Johnson's Ad26.COV2.S, Sputnik-V, and adjuvanted recombinant protein nanoparticle Novavax[10], are leading the remarkable research and development efforts against COVID-19[11]. At present, 242 vaccines targeting COVID-19 are undergoing clinical research, involving 46 in Phase III and 46 in Phase IV trials[12]. Vaccination has been recognized as a crucial preventive measure against exposure to SARS-CoV-2 infection. Improving and optimizing existing vaccines and developing new ones will be highly efficacious against COVID-19, aiding in ending the pandemic.

Disinfection as a preventive measure

Dynamic disinfection: Researchers have discovered the presence of SARS-CoV-2 on surfaces particularly in hospitals where COVID-19 patients were admitted, indicating the need for surface disinfection[13]. Adequate ventilation and air purification systems can reduce the risk of COVID-19 transmission. Ultraviolet (UV) light is a traditional method for air

Table 1 Vaccine information

Vaccine classification	Name of vaccine approved by the WHO	Vaccine research and development unit
Inactivated vaccine	BBIBP-CorV	Sinopharm
Inactivated vaccine	CoronaVac	Sinovac Biotech
Inactivated vaccine	COVAXIN	Bharat Biotech International
Viral vector vaccine	AZD1222	AstraZeneca-University of Oxford
Viral vector vaccine	Ad26.COV-2-S	Johnson & Johnson
Viral vector vaccine	COVISHIELD	Serum Institute of India
Protein subunit vaccine	NVX-CoV2373	Novavax
Protein subunit vaccine	COVOVAX	Serum Institute of India
mRNA vaccine	BNT162b2	Pfizer-BioNTech
mRNA vaccine	mRNA-1273	Moderna

WHO: World Health Organization; mRNA: Messenger ribonucleic acid.

disinfection, which is simple to be used as an effective method against airborne bacteria. However, its prolonged exposure can irritate human eyes, skin, and respiratory mucosa, leading to the decreased white blood cell count and the increased risk of skin cancer[14]. Therefore, UV disinfection should be applied only in unoccupied areas. When inhaled, ozone competes with hemoglobin for oxygen in the bloodstream and harms human health, making it inappropriate for air disinfection in occupied spaces. Disinfectant sprays provide temporary effects on contaminated environments. Cleanrooms and central air conditioning systems with filtration are expensive and challenging to implement widely. Air disinfection machines utilize low-ozone, high-intensity UV lamps, electrostatic adsorption, negative ion generators, and air filtration systems to eliminate bacteria collectively in the air. Dynamic air disinfection, conducted without disrupting regular operations, helps maintain air cleanliness and reduces airborne microbial content[15]. This method has been proved more effective than static UV disinfection. Air disinfection machines are harmless to humans, can operate in occupied spaces, are user-friendly, and are proper for utilization in grassroots-level hospitals, contributing to preventing and controlling COVID-19 infections[16].

Precise disinfection: Disinfection can promptly cut off the transmission routes of infectious diseases, while it should be conducted with scientific precision. If disinfection is not standardized and methods are incorrect, it may not control the epidemic and may even lead to additional health safety issues. Different disinfection methods are employed for various contaminants of the novel coronavirus, targeting indoor air, pollutants, surfaces of floors and walls, object surfaces, clothing and textiles, dining utensils, skin mucous membranes, and transportation tools. To date, numerous studies have concentrated on disinfection techniques. These studies mainly included assessment through air sampling before and after disinfection, as well as surface sampling of objects, to verify the effectiveness of the disinfection process[17]. Excessive disinfection poses risks to personal safety. Spraying disinfectants on individuals without protective gear can lead to several diseases. There are potential risks if individuals inhale disinfectants or are repeatedly spread with them all over their bodies[18], which can also result in environmental pollution[19]. Microorganisms may develop drug resistance, diminishing the sterilization effect, and the chemical residues left in the environment are new sources of pollution, disrupting ecological balance if excessive disinfection is used.

Cold sterilization: Cold chain systems play a vital role in maintaining food quality, extending shelf life, and reducing food waste. During the pandemic, the food cold chain systems could potentially serve as a medium for the transmission and infection of SARS-CoV-2. Despite the likelihood of foodborne transmission being lower than other transmission pathways, considering the substantial quantity of refrigerated foods transported across different countries and regions, cold chain transportation remains a significant risk factor that should not be overlooked. SARS-CoV-2 may persist on the surfaces of packaged food under cold processing and refrigeration temperatures for up to 60 days[20]. Several imported cold chain products were tested positive for COVID-19 at Chinese customs. In June 2020, sealed packaging containing salmon in a cold storage area outside the Xinfadi Market in Beijing, China, was tested positive for SARS-CoV-2 RNA[21]. Since the initial discovery of COVID-19 related to cold chain incidents in 2020, Guangzhou has reported a total of 283 COVID-19-positive cases related to the cold chain[22]. Therefore, there is a need for virus sterilization methods specific to the cold chain environment to maintain its safety[23]. At present, customs primarily use chemical disinfection as a sterilization method. Additionally, high-intensity ultraviolet-C irradiation has proven effective in deactivating SARS-CoV-2 at typical cold chain temperatures[24]. Analyzing the resistance data of coronaviruses in the environment revealed the importance of disinfecting the patient's living environment, potentially contaminated items, as well as waste, sewage, and excreta.

Management of talent development

Monitoring and controlling nosocomial infections are crucial tasks for hospitals; individuals must implement effective surveillance and control plans tailored to their circumstances. Typically, epidemiologists, supervisors, and infection control personnel should collaborate to implement scientific monitoring and control measures. The current pandemic has revealed the persistent vulnerability of public health systems, particularly due to insufficient public health professionals, especially at senior levels. The imbalance in the development between medical and public health systems has resulted from the emphasis on medical treatment over prevention. This has led to a weakening of professional value, declining recognition, rapidly diminishing awareness, inadequate attractiveness of the profession, and a significant talent drain. These issues underscore the severe deficiency in the training and retention of public health professionals. Therefore, cultivating and managing public health talent in hospitals are exceedingly critical. To enhance the effectiveness of public health talent's cultivation and management, we should emphasize the principle of prevention as the primary focus, combined with treatment. Allocating substantial resources and investments in preventive medicine education is crucial for fostering public health professionals[25]. Establishing a comprehensive research system across the entire spectrum, including health assessment and exposure evaluation, rapid disease diagnosis and forecasting, standardized clinical research and trials, vaccine development and evaluation, population control strategies, health management, and regulatory formulation, is crucial. Additionally, building an emergency response mechanism and promptly assembling specialized public health emergency teams comprising epidemiologists, infection control specialists, and public health managers are essential[26]. Given the analysis of the pathogenic mechanisms, transmission routes, and resistance characteristics of the novel coronavirus, prompt disinfection of places before viral transmission is imperative to sever the transmission pathway. Additionally, routine disinfection of air and items in contact with infected individuals by staff members is essential. Environmental areas where cases have been isolated, living quarters, transportation, and objects touched by the infected should undergo specific disinfection. Professional disinfection personnel must disinfect and handle the contracted areas after thorough isolation. The disinfected regions and contact points must be entirely free of viruses.

Furthermore, encouraging interdisciplinary collaboration and exchanges among professionals from diverse disciplines, such as medicine, public health, and psychology is critical[27]. Engaging in co-designed and executed training programs, along with regular multidisciplinary seminars and case analysis sessions for senior management and potential leaders, are essential components of leadership and crisis management training[28]. Periodically evaluating the performance of management personnel and providing feedback and advice may assist in enhancing their decision-making and team management skills. Providing scholarships, research opportunities, and domestic as well as international exchange programs for public health workers may encourage innovative thinking and the application of new technologies. This fosters their enthusiasm for learning and innovation. Moreover, prioritizing management of mental health and professional burnout is also essential. Providing psychological support and stress management training for frontline medical and public health workers, devising rational work schedules, and implementing rotation systems may prevent professional burnout[29]. Additionally, ensuring adequate and equitable resource allocation, judicious distribution of human resources, and ensuring sufficient professional personnel in critical positions are vital. Adapting training content and methods flexibly based on changes in the epidemic and new characteristics of public health challenges may enhance the adaptability and efficiency of public health personnel. These strategies enable effective training and management of hospital public health personnel to confront the challenges posed by the novel coronavirus and prepare for the future public health crises.

TREATMENT MEASURES

Therapies targeting inflammatory cytokines

During SARS-CoV-2 infection, some cases with chronic illnesses may experience 'cytokine storm'. This severe immune response involves releasing numerous cytokines in the body, resulting in intense inflammation, which can lead to multi-organ failure even death[30]. Therefore, managing the cytokine storm is a crucial aspect of COVID-19 treatment. There are several treatment measures for addressing cytokine storm and inflammatory response in COVID-19 patients. Corticosteroids, such as dexamethasonecan, suppress the inflammatory response, reducing the mortality rate in patients requiring mechanical ventilation, and they are utilized to treat severe and critical COVID-19 cases[31]. Some cytokine inhibitors (*e.g.*, tocilizumab and sarilumab), which are interleukin-6 (IL-6) receptor antagonists, can be used to treat critically ill COVID-19 patients, improving survival rates[32]. These medications can inhibit the IL-6 signaling, thereby alleviating inflammation. Baricitinib, a JAK inhibitor, can block inflammatory signal transduction and is used in the treatment of early-stage COVID-19 patients[33]. Studies demonstrated that mesenchymal stem cell therapy might be beneficial for treating severe COVID-19 patients, particularly in suppressing inflammation and promoting tissue repair. However, the application of mesenchymal stem cell therapy is still under investigation, and its safety and efficacy require further clinical validation[34]. Additionally, healthcare professionals need to consider specific factors, including the severity of patients' condition and existing underlying diseases. Geetha *et al*[35] studied and demonstrated a positive correlation between elevated inflammatory markers, including C-reactive protein, ferritin, and D-dimer, and the rate of invasive and non-invasive mechanical ventilation among COVID-19 patients with chronic kidney disease (CKD), suggesting that these inflammatory biomarkers may be used as clinical tools to guide the diagnosis and management amongst stage IIIb-V CKD patients with COVID-19 disease. Their specific treatment measures should be conducted based on clinical guidelines and professional medical advice to ensure safety and efficacy of treatment.

Other new drug treatments

Remarkably, with the ongoing development of new research findings and clinical practices, an increasing number of novel methods and medications for treating COVID-19 have emerged (Table 2). Consequently, healthcare professionals need to stay updated with the latest research advancements and guidance from health authorities to promptly adjust their treatment protocols. Remdesivir, a broad-spectrum antiviral drug initially used for treating Ebola virus infection, has exhibited to inhibit SARS-CoV-2 replication and reduce viral load when administered preventively or in the early stages[36]. Oral antiviral drugs, such as Molnupiravir and Paxlovid, significantly reduce the rates of hospitalization and mortality, and they can be utilized for treating mild-to-moderate COVID-19 cases, particularly for treating patients who are at the high-risk of severe illness[37]. Monoclonal antibodies (*e.g.*, Bamlanivimab and Etesevimab) used in early infection prevent virus binding to host cells, impeding viral replication[38]. Another combination, Casirivimab plus Imdevimab (REGN-COV2), is used for treating mild-to-moderate COVID-19 cases. Immunosuppressants, such as cyclosporine and other immunomodulators, may assist in tempering an overactive immune system, while their usage requires careful consideration and guidance by healthcare professional[39]. Convalescent plasma therapy, using the plasma from recovered individuals containing antibodies capable of neutralizing the virus, is also noteworthy, which may alleviate the cytokine storm by removing inflammatory mediators and cytokines from the patient's blood[40].

When using these medications, individual patient differences, drug tolerability, potential complications, and drug interactions must be thoroughly considered. Importantly, all new therapeutic interventions should be employed in appropriate clinical trials and in accordance with recommendations from national health authorities, following the latest guidelines from international organizations, involving the WHO. As the situation evolves and research progresses, treatments for COVID-19 will continue to be updated and refined.

CONCLUSION

During the COVID-19 pandemic, healthcare-associated infections posed a significant challenge in the hospital. COVID-19 could spread through direct contact with the patient's blood, body fluids, respiratory droplets, and virus-contaminated surfaces. However, the combination of high population density and a large susceptible population has led to hospitals frequently encountering a significant number of diagnosed COVID-19 cases and asymptomatic carriers. This poses a serious threat of nosocomial infections during the pandemic. Therefore, reducing infections in healthcare facilities remains an urgent and formidable task for healthcare providers.

This study explored some primary strategies for preventing and controlling hospital-acquired COVID-19 infections (Figure 1). Firstly, vaccination plays a crucial role in preventing and controlling COVID-19, reducing the rates of hospitalization, mortality, and infection. Secondly, effective disinfection methods also play a vital role, interrupting the transmission chain through dynamic, targeted, and low-temperature disinfection approaches. Thirdly, nosocomial infection management is a also critical component of healthcare quality control. Particularly in high-risk populations with inadequate preventive measures, early detection of mild and asymptomatic cases is paramount in the current stage of epidemic prevention and control. Adherence to safety distances, isolation measures, adequate supplies, and a well-functioning healthcare system are crucial preventive measures for hospital-acquired infections. When treating infected patients, healthcare workers must consider patients' specific conditions, including illness severity, potential complications, and underlying health conditions. These treatment measures should be guided by clinical guidelines and professional medical advice to guarantee patient safety and treatment effectiveness. As scientific technologies advance, strategies for COVID-19 prevention and control, as well as novel public health interventions, will continue to evolve and enhance. Integrated prevention and treatment approaches will be instrumental in managing nosocomial infections during the ongoing COVID-19 pandemic.

Table 2 Drugs information

Therapeutic drugs	Mechanism
Remdesivir	Inhibiting virus replication
Molnupiravir	Inhibiting virus replication
Paxlovid	Inhibiting virus replication
Bamlanivimab/Etesevimab	Blocking the binding of viruses to host cells
Casirivimab/Imdevimab	Blocking the binding of viruses to host cells
Cyclosporin	Inhibiting overactivated immune system
Recovery period plasma therapy	Antibodies that neutralize viruses

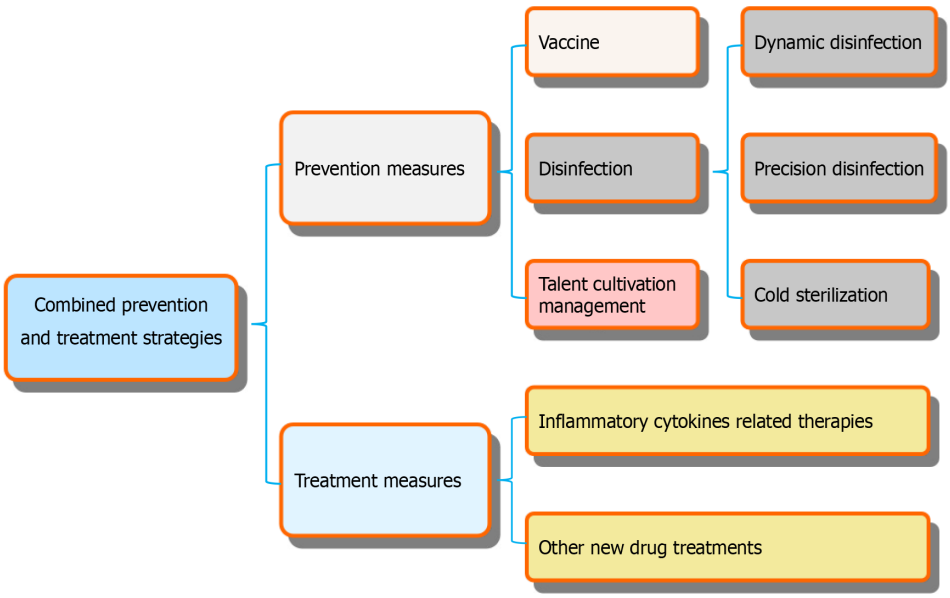

Figure 1 Combined prevention and treatment strategies are essential for preventing and controlling nosocomial infections during the coronavirus disease 2019 pandemic.

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FOOTNOTES

Author contributions: Wang MK and Yang JS conceptualized, designed, and revised the manuscript; Liu JW drafted the manuscript; Li YY collected the literature. All authors have read and approved the final manuscript. Both Wang MK and Yang JS have conceptualized, proposed, designed, and supervised the whole process of the article, and played important and indispensable roles in the manuscript preparation and revision as the co-corresponding authors. Wang MK applied for and obtained the funds for this research project. Wang MK conceptualized, designed, revised and supervised the whole process of the project. Yang JS was instrumental, conceptualized, and revised the manuscript. This collaboration between Wang MK and Yang JS is crucial for the publication of this manuscript and other manuscripts still in preparation.

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ORCID number: Jing-Wen Liu 0009-0006-2723-620X; Yue-Yue Li 0009-0000-6735-8128; Ming-Ke Wang 0000-0001-9918-0491; Ji-Shun Yang 0000-0001-7160-706X.

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REVIEW

Pathogenesis and clinical features of severe hepatitis E virus infection

László Orosz, Károly Péter Sárvári, Áron Dernovics, András Rosztóczy, Klára Megyeri

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László Orosz, Károly Péter Sárvári, Áron Dernovics, Klára Megyeri, Department of Medical Microbiology, University of Szeged, Szeged 6720, Csongrád-Csanád, Hungary

András Rosztóczy, Department of Internal Medicine, Division of Gastroenterology, University of Szeged, Szeged 6725, Csongrád-Csanád, Hungary

Corresponding author: Klára Megyeri, MD, PhD, Associate Professor, Department of Medical Microbiology, University of Szeged, Dóm tér 10, Szeged 6720, Csongrád-Csanád, Hungary. megyeri.klara@med.u-szeged.hu

Abstract

The hepatitis E virus (HEV), a member of the *Hepeviridae* family, is a small, non-enveloped icosahedral virus divided into eight distinct genotypes (HEV-1 to HEV-8). Only genotypes 1 to 4 are known to cause diseases in humans. Genotypes 1 and 2 commonly spread *via* fecal-oral transmission, often through the consumption of contaminated water. Genotypes 3 and 4 are known to infect pigs, deer, and wild boars, often transferring to humans through inadequately cooked meat. Acute hepatitis caused by HEV in healthy individuals is mostly asymptomatic or associated with minor symptoms, such as jaundice. However, in immunosuppressed individuals, the disease can progress to chronic hepatitis and even escalate to cirrhosis. For pregnant women, an HEV infection can cause fulminant liver failure, with a potential mortality rate of 25%. Mortality rates also rise amongst cirrhotic patients when they contract an acute HEV infection, which can even trigger acute-on-chronic liver failure if layered onto pre-existing chronic liver disease. As the prevalence of HEV infection continues to rise worldwide, highlighting the particular risks associated with severe HEV infection is of major medical interest. This text offers a brief summary of the characteristics of hepatitis developed by patient groups at an elevated risk of severe HEV infection.

Key Words: Hepatitis E virus; Cirrhosis; Acute-on-chronic liver failure; Pregnancy; Immune dysfunction; Open reading frames 1-4

Core Tip: Hepatitis E virus (HEV) typically causes a self-limiting infection, but it can establish a persistent infection progressing into chronic hepatitis, cirrhosis or acute liver failure, particularly in individuals with compromised immune systems and preexisting liver diseases. In recent decades, the prevalence of hepatitis E has increased dramatically, and this trend continues unabated. Thus, a better understanding of the pathogenesis of hepatitis E and the development of more effective prevention and treatment strategies have become an urgent medical problem. We herein discuss the major features of HEV, the conditions predisposing to severe hepatitis E, and the underlying pathological immune processes.

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INTRODUCTION

The hepatitis E virus (HEV), a non-enveloped virus characterized by its icosahedral capsid symmetry and approximately 27-34 nm size, belongs to the *Hepevirus* genus within the *Hepeviridae* family. Its genome is a positive-sense, single-stranded RNA strand of roughly 7.2 kb[1,2]. There are eight known genotypes of HEV, but genotypes 1-4 account for most human diseases[3]. HEV primarily spreads through the fecal-oral route, with contaminated water and undercooked meat being common infection sources[3,4]. Typically, HEV infections are self-limiting and resolve naturally within 6–8 wk[4]. However, in immunocompromised individuals, HEV infection can become chronic[3,4]. Persistent infections could lead to serious conditions such as liver cirrhosis, liver failure, and hepatocellular carcinoma (Figure 1)[5-11].

The interplay between HEV infection and conditions like liver cirrhosis, pregnancy, and immunosuppression warrants significant attention in hepatology. Liver disease progression is profoundly impacted when individuals with cirrhosis contract HEV, resulting in a greater risk of acute-on-chronic liver failure (ACLF). This necessitates careful monitoring and proactive strategies for HEV detection, prevention, and treatment among these at-risk individuals.

Pregnant women, particularly in their third trimester, also face a significant risk of severe complications and death from HEV infection[12]. The underlying factors for these complications during pregnancy remain unknown, highlighting an urgent need for more research in this area.

Immunosuppressed individuals, including organ transplant recipients and those with human immunodeficiency virus (HIV), elevate the possibility of chronic HEV infection and subsequently developing liver cirrhosis.

In summary, a strong connection exists between HEV infection and liver cirrhosis, especially in pregnant and immunosuppressed individuals. As such, acknowledging HEV infection as a potential risk factor and implementing prevention, detection, and treatment strategies are fundamental, particularly for those with liver disease or those undergoing immunosuppressive treatment. This review offers a broad perspective on the relationship between HEV infection and liver cirrhosis by consolidating available data on this matter.

SEARCH STRATEGY AND SELECTION CRITERIA

We carried out a literature search through the PubMed database using the following search terms: “hepatitis E” limited to “open-reading frame”, “replication”, “innate immune response”, “cytokine”, “humoral immune response”, “cellular immune response”, “acute-on-chronic liver failure”, “pregnancy”, “immunosuppression”, and “cirrhosis”. We excluded articles pertaining to diagnostics, therapy, animal studies, and case reports already covered in other included studies. From the 5744 articles initially found, we selected 149 for our study. All chosen materials were in English.

THE GENERAL ORGANIZATION OF HEV

The RNA genome of HEV is capped at the 5′ end and polyadenylated at the 3′ end. It contains three slightly overlapping open reading frames (ORF) 1–3 along with a recently discovered ORF4[1,13,14]. Additionally, infected cells produce a 2.2 sub-genomic RNA from which ORF2 and ORF3 are translated[1].

ORF1

The 5079 base pairs (bp) ORF1 sequence encodes a polyprotein comprised of 1693 amino acids (aa) that weighs 190-kDa [15]. This ORF1 sequence encodes nonstructural proteins that are enzymatically active, including methyltransferase, papain-like cysteine protease (PCP), RNA helicase, RNA-dependent RNA polymerase (RdRp), macro domains or X/Y domains, and a hypervariable region. These proteins play crucial roles in RNA synthesis, RNA capping, RNA unwinding, tRNA metabolism, transcription, and replication[16].

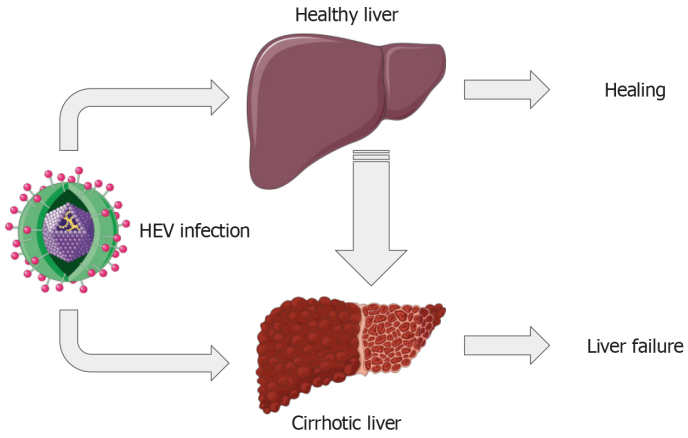

Figure 1 Varying consequences of hepatitis E infection in individuals with healthy and distressed livers. HEV: Hepatitis E virus. Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/Licenses/by/3.0/).

The methyltransferase, located on ORF1 polyprotein positions 60 to 240, and a 110-kDa protein (on ORF1 polyprotein positions 1 to 979) with guanyltransferase and guanine-7-methyltransferase activity are involved in viral RNA capping (m7G cap: 27–35 nucleotides at the 5′ end). This is because the 7-methylguanine cap is critical for HEV infectivity[17,18].

The PCP (potentially located on ORF1 polyprotein positions 433 to 592) may also inhibit cellular antiviral immune function[19]. Meanwhile, RNA helicase (on ORF1 polyprotein positions 960 to 1204) is a member of the 5′→ 3′ class of the superfamily 1 of helicases and plays a vital role in HEV RNA replication[20,21].

Furthermore, RdRp is found on ORF1 polyprotein positions 1207 to 1693 and is an essential enzyme for RNA replication[22]. Macro domain proteins (on ORF1 polyprotein positions 775 to 960) hydrolyze adenosine diphosphate (ADP)-ribose 1″-phosphate and might contribute to RNA replication, posttranslational modification, and cellular apoptosis[23].

Lastly, the Polyproline region or Hypervariable region has several potential functions, such as providing peptide cleavage sites, areas modified by enzymes, and sites that can bind to proteins, nucleotides, and metal ions. This suggests a potential role in viral replication[24].

ORF2

ORF2, featuring 1980 bp, encodes the capsid protein, which comprises 660 aa with an approximate molecular weight of 72 kDa[25]. ORF2 of the HEV genotype 3 consists of three sections: The N-terminal domain (aa 1–111), virus-like particle (VLP) (aa 112–608), and C-domain (aa 609–660).

The VLP can be further subdivided into the shell domain (S, aa 129–319), the middle domain (M, aa 320–455), and the protruding domain (P, aa 456–606)[26,27]. Each VLP subdivision contains neutralization epitopes recognized by anti-HEV immunoglobulins (IgM and IgG) responsible for attaching the virion to susceptible cells[28].

A separate product can also be identified-a glycoprotein weighing approximately 88 kDa with three potential glycosylation sites. This can be transferred through the endoplasmic reticulum and expressed on the surface[29].

Three variations of ORF2 from the HEV genotype 3 have been noted: ORF2i (the infectious form), ORF2g (the glycosylated and secreted version), and ORF2c (the cleaved and secreted type). ORF2c is theorized to be a cleavage product of the ORF2g protein.

All ORF2 proteins possess three potential N-glycosylation sites and several O-linked glycosylation sites. ORF2g and ORF2c are secreted and glycosylated (glycosylation sites: N1 and N3 in ORF2g/c) but do not associate with infecting virions. In contrast, ORF2i is not glycosylated but is the only version packaged into infectious particles[30].

Lastly, ORF2s is the secreted form of ORF2, which is glycosylated and does not contain the binding site of the cellular receptor[31].

ORF3

The smaller third ORF is located at the end of ORF1, and it overlaps ORF2 at the 5′ end[2]. It is translated from bicistronic sub-genomic RNA. This ORF, termed ORF3, encodes a protein consisting of 123 aa known as Vp13, a non-glycosylated protein with an approximate size of 13.5-kDa[1,2].

Vp13 is notable for containing two proline-rich domains (P1 and P2) and two strong hydrophobic regions (D1 and D2) in the N-terminal half. It can be phosphorylated at a serine residue (ser-80), which is crucial for its interaction with the capsid protein[1,32]. This phosphorylation may be necessary for *in vivo* replication. Additionally, the palmitoylation of Vp13 plays a significant role in the release of virions[33].

Vp13 is known to interact with the cell's cytoskeleton and aids in binding to microtubules[32,34]. The P2 domain specifically has two PXXP motifs, where P and X designate proline and an unspecified amino acid, respectively. PXXP motifs are instrumental in the interaction of the P2 domain with the SH3 domains of cellular proteins, such as the α-1-microglobulin and bikunin precursor protein. This interaction can activate the mitogen-activated protein kinase, which

could disrupt cellular signal transduction[35,36].

ORF3's functionality extends to its binding with hemopexin, a protein that protects against oxidative damage. An HEV infection is linked to a decline in the serum levels of hemopexin, which indicates the pivotal role of ORF3 in infection[37]. Vp13 also functions as a class I viroporin–its two PXXP motifs form an ion channel instrumental in viral discharge[38].

ORF4

In a recent discovery, another ORF called ORF4 was found in HEV genotype 1[13]. This ORF4 transcription is only noticeable under the stress of the endoplasmic reticulum and plays a key role in viral replication by maintaining the proper function of RdRp[14].

Replication of HEV

The hepatocyte is the main cell type wherein HEV replicates, though HEV can also replicate in other cells and tissues like monocytes, the spleen, lymph nodes, and the small intestine[39]. The virus usually spreads through fecal-oral transmission and reaches the hepatocytes *via* the bloodstream. A cellular receptor for viral binding has not been identified yet. Still, non-enveloped virions need heparan sulfate proteoglycan for attachment, whereas quasi-enveloped virions attach to the cell surface independently[40].

Research by Holla *et al*[41] suggests that the virions may enter host cells through a process known as dynamin-2-dependent clathrin-mediated endocytosis[41]. The uncoating of HEV requires a low pH. The virus's positive-sense RNA genome is subsequently released into the cytosol and serves as the template for the translation of ORF1[42].

Transcription begins when RdRp binds to the 3′ untranslated region of the viral RNA to produce the negative-sense intermediate RNA. This serves as a template for synthesizing positive-sense RNA and is translated into ORF2 and ORF3 proteins[43]. Structural proteins might be expressed from two sub-genomic RNAs (3.7 kb and 2.0 kb). The ORF2 protein packages the genomic positive-sense RNA into progeny virions[30].

The HEV virions assembled in this manner bud into the lumen of multivesicular endosomes, thus acquiring a quasi-envelope. These loaded endosomes then move to the cytoplasmic membrane, fusing with it and releasing the quasi-enveloped virions into the space outside the cells.

In the bile canaliculi, these quasi-enveloped HEV (eHEV) virions lose their envelopes to the bile and convert into non-enveloped particles, though a small number of eHAV are released into the blood *via* the hepatocytes' basolateral side. The removal of the lipid layer makes the eHEV particles more infectious[44].

THE MAIN ELEMENTS AND CHARACTERISTICS OF THE IMMUNE RESPONSE ELICITED BY HEV

The innate immune response

Innate immune cells interact with HEV by using their pattern recognition receptors (PRRs), such as Toll-like receptor (TLR) 2 and TLR4, which recognize the capsid. Other receptors, like TLR7/8, Retinoic Inducible Gene-I (RIG-I)-like receptors, and Melanoma Differentiation-Associated Protein 5 (MDA5), attach to the viral RNA[45]. It has been shown that TLR2, 3, 4, 7, and 8 expression levels heighten in the peripheral blood mononuclear cells (PBMC) of HEV patients[46,47]. Studies also suggest that A549 cells show increased PRR levels following HEV infection[48].

The interaction of HEV with TLRs, RIG-I, and MDA5 activates the interferon regulatory factor (IRF) 3 and nuclear factor-κB (NF-κB) transcription factors through signaling pathways that involve Toll/interleukin (IL)-1 receptor domain-containing adaptor inducing interferon (IFN) β, tumor necrosis factor (TNF) receptor-associated factor (TRAF) 3, TRAF6, myeloid differentiation primary response 88, and mitochondrial antiviral-signaling protein (MAVS) adaptors[47,48]. These activated transcription factors, in turn, stimulate the transcription of numerous pro- and anti-inflammatory cytokine genes.

During self-limited acute HEV infection, among the pro-inflammatory cytokines and chemokines, the levels of TNF-α, IFN-α, IFN-ω, IFN-β, IFN-γ, IFN-λ, IL-1β, IL-4, IL-6, IL-18, C-X-C motif chemokine ligand (CXCL) 8 (IL-8), C-C motif chemokine ligand (CCL) 5 protein [Regulated upon activation, normal T cell expressed and secreted (RANTES)], interferon-stimulated gene (ISG) 15, and ISG20 were increased in sera or PBMC obtained from HEV-infected patients[47,49,50]. In hepatocytes and enterocytes, HEV virions or RNA triggered the production of IFN-β, IFN-λ1, IFN-λ2 and IFN-λ3 in a cell type-dependent manner. Human and swine hepatocytes mainly secreted type I IFNs, whereas enterocytes predominantly produced type III IFNs[51,52]. Interestingly, IRF3 and IRF7 were essential for the HEV genomic RNA-mediated induction of IFNs, while the cellular RNA sensing pathways were not required[52].

IFNs bind to their corresponding receptors and activate the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway, stimulating the expression of several hundred genes, whose products exert antiviral, immunomodulatory, and antiproliferative actions. IFNs induced by HEV infection increase the expression of ISG15, IFN-α inducible protein 6, MX1, 2′, 5′-oligoadenylate synthetase 1, and interferon-induced proteins with tetratricopeptide repeats 2, all of which belong to the IFN-stimulated genes[48,52]. All types of IFNs inhibit HEV replication directly, but HEV can impair the function of the IFN system.

HEV encodes a microRNA (miRNA), HEV-miRNA-A6, which can inhibit the phosphorylation of IRF3, thereby reducing the production of type I IFNs[53]. Moreover, HEV ORF-1 interferes with the recognition of viral RNA by RIG-I and impairs the activation of the JAK-STAT signaling pathway by inhibiting STAT-1 activation and nuclear translocation [54,55]. Hence, ORF-1 inhibits the transcriptional activation of ISGs and the antiviral effect of type I IFNs (IFN-α, IFN-β and IFN-ω)[54,55]. Consequently, HEV induces the synthesis of numerous pro- and anti-inflammatory cytokines while inhibiting the production and effects of type I interferons, resulting in a cytokine environment with a weak antiviral

effect. This environment is unable to effectively control virus replication in the early stages of infection.

The humoral immune response

During an acute, self-limited infection, anti-HEV IgM is found concurrently with a rise in transaminase levels in the bloodstream and starts to decrease after recovery. On average, the persistence of anti-HEV IgM lasts for around 20 wk, although in some cases, it can be detected even after 3 years[56-58]. The reasons for this prolonged presence are still unclear. In individuals having HIV, those who have undergone transplantation, patients with cancer and those who are immunosuppressed, the emergence of HEV-specific IgM may take months[57-59].

During the early stage of infection, anti-HEV IgA can also be detected in the bloodstream for at least 4 months[60]. Following the emergence of IgM, anti-HEV IgG becomes noticeable in the bloodstream. Its titer and avidity increase and can persist for at least 5 years before gradually decreasing[58,61-63]. HEV-specific antibodies might have a potentially protective role by neutralizing effects and initiating some antiviral immune mechanisms.

Immunodominant conformational and non-immunodominant linear epitopes have been identified on the ORF2 protein of HEV[64-67]. These neutralizing antibodies work by inhibiting the virus from binding to cell surface receptors and entering the cells. Interestingly, while immune sera can neutralize virion infectivity in feces[68], there's been no such neutralizing effect on HEV particles in the bloodstream. The blood-circulating HEV particles are quasi-enveloped, surrounded by a host cell-derived membrane lacking viral proteins[69]. Thus, neutralizing antibodies cannot bind with these eHEV particles. As a result, less than 10% of HEV virions bind to circulating antibodies, partially restricting antibody-mediated neutralization[68].

HEV-specific non-neutralizing antibodies may also contribute to immune defense through other mechanisms like epitope unmasking, intracellular neutralization, antibody-dependent cellular cytotoxicity, activation of the classical complement pathway, and phagocytosis[70]. The role of these non-neutralizing antibodies in immune protection against HEV, however, remains unclear. Although humoral immunity helps slow down the rate of viral replication and reduce the severity of symptoms, it is not enough for full protection. Therefore, breakthrough infections can still occur in both immunocompetent and immunosuppressed individuals with measurable humoral immunity levels.

The cellular immune response

HEV-specific cellular immune responses have been extensively studied by analyzing the number, immune phenotype, and function of PBMC or hepatic immune cells. Stimulation of PBMC with a HEV genotype 3a peptide library, encompassing 616 peptides spanning ORF1-3, or a recombinant capsid protein, resulted in robust T-cell responses in seropositive healthy individuals, as evidenced by IFN-γ production[71-73]. While peptides derived from all three ORFs triggered strong cellular immune responses, ORF2 was identified as carrying the immune-dominant epitopes of HEV [71]. However, both $CD4^+$ and $CD8^+$ T-cell responses against peptides from ORF2 and ORF3 were absent, and IFN-γ production was diminished in organ transplant recipients with chronic hepatitis E[73]. The activation, proliferation, and cytokine secretion of lymphocytes is regulated by inhibitory receptors like programmed cell death protein 1 (PD1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA4). Blockade of the PD1 and CTLA4 pathways using anti-PDL1 and anti-CTLA4 antibodies restored the proliferative responses of $CD4^+$ and $CD8^+$ lymphocytes stimulated with an ORF2 and ORF3 peptide pool[73].

Kemming *et al*[74] identified several HEV-specific $CD8^+$ T-cell epitopes restricted by nine different HLA class I alleles, with 17 and eight out of 25 epitopes located on the ORF1 and ORF2 proteins, respectively[74]. The immune-dominant cytotoxic T lymphocyte (CTL) epitopes of HEV were found within the ORF2 protein[74]. During self-limited acute infection, elevated expressions of activation and proliferation markers (CD38 and Ki67), Tbet transcription factor, and granzyme B were observed, indicating the development of an intense effector $CD8^+$ T-cell response[74]. In the recovery phase, these markers were downregulated, and a stable memory cell pool was established[74]. In contrast, patients with chronic hepatitis E exhibited significantly diminished $CD8^+$ T-cell responses. These $CD8^+$ T-cells co-expressed activation and proliferation markers along with the PD1 inhibitory receptor, reflecting their terminally exhausted phenotype and profound functional deterioration[74]. Thus, HEV-specific $CD8^+$ T cells are key cellular elements of protective immunity, playing an essential role in virus elimination.

Another study revealed higher numbers of $CD4^+CD25^+Foxp3^+$ and $CD4^+CD25^-Foxp3^+$ regulatory T-cells in the blood of patients with self-limited acute HEV infection and recovered individuals compared to seronegative controls[75]. Increased IL-10 production was also detected in PBMC stimulated with recombinant ORF2 in these patient groups[75]. Transient differences were observed in the number and activation state of natural killer (NK) and NKT cells, as well as in the cytotoxic activity of NK cells in the peripheral blood of acute hepatitis E patients[76].

El Costa *et al*[77] compared the $CD8^+$ T-cell responses between asymptomatic and symptomatic hepatitis E patients [77]. Symptomatic elderly patients exhibited a comprehensive global expansion of an activated effector memory $CD8^+$ T-cell compartment, consisting of both HEV genotype 3-specific and non-specific cell populations[77]. The cells isolated from symptomatic elderly patients were HLA-DR/CD38/PD1 triple-positive, showed increased C-X-C motif chemokine receptor (CXCR) 3, IL-4, and granzyme B expression, and decreased IFN-γ production[77]. The levels of CXCL9 and CXCL10, known ligands of CXCR3, were also markedly elevated in symptomatic patients[77]. Further investigations of immune cell phenotypes in biopsy specimens obtained from patients with HEV-induced acute liver failure revealed the accumulation of activated $CD8^+$ T-cells containing granzyme in the liver[78]. These studies, which highlight the emergence of a $CD8^+$ T-cell population with high cytotoxic potential and the ability to infiltrate the liver during symptomatic infections, support the idea that liver damage caused by HEV infection is mediated by pathological immune processes[77,78].

THE MAIN FEATURES OF SEVERE HEPATITIS E

HEV infection and acute-on-chronic liver failure

ACLF is a disorder marked by sudden liver damage, evident through jaundice and coagulopathy. ACLF differs from chronic hepatic decompensation in several ways. Notably, liver failure and the resulting organ dysfunction progress faster in ACLF. Furthermore, the 3-month death rate during ACLF is much higher than predicted in chronic hepatic decompensation cases[79,80]. Usually, ACLF develops after cirrhosis of the liver, with additional factors possibly exacerbating the liver damage. These can include co-infection with other forms of viral hepatitis (like HEV), ischemic hepatitis, alcoholic hepatitis, drug-induced hepatitis, and metabolic dysfunction-associated steatotic liver disease and steatohepatitis (MASLD/MASH). Recent studies suggest that a combination of viral hepatitis and surgery are the primary factors causing ACLF[81].

The transition from stable cirrhosis to ACLF is triggered by the production of pro-inflammatory cytokines due to a systemic inflammatory response. Inflammatory mediators have been shown to enhance hyperammonemia's induction of encephalopathy[82]. Previous studies have noted a decrease in peripheral $CD4^+$ T-cell levels and an increase in $CD8^+$ T-cell levels in individuals with HEV infection, causing an alteration in the CD4/CD8 ratio[78]. A systemic inflammatory response also considered a negative prognostic factor, is associated with the development of encephalopathy, thereby reducing the potential for successful organ transplantation[83]. Moreover, hepatic synthesis often weakens, causing hypoalbuminemia, which subsequently results in edema and ascites. Hyperbilirubinemia and clinical jaundice are typically unavoidable, while thrombocytopenia can cause hemorrhagic diathesis (Figure 2). Altogether, given the well-known link between HEV infection and ACLF, it is essential to take proactive steps to manage this public health issue and shield vulnerable populations from the severe implications of liver complications due to HEV.

HEV infection and pregnancy

Pregnancy can complicate the course of an infection, making it more severe. Changes in hormone concentrations and immune responses induced by pregnancy can affect the immune system's ability to fight infections like hepatitis E. Other than these factors, specific viral elements have been identified that could exacerbate the infection's progression (Figure 3).

The severe liver damage caused by a HEV infection during pregnancy can be attributed to unclear pathophysiological mechanisms. Past studies have suggested that the unique conditions in pregnant women, like altered immune responses, hormone levels, and viral aspects, such as the diversity and changes in the HEV genome, could potentially affect the disease's severity[84]. Throughout pregnancy, the mother's immune system is challenged to maintain a robust response to ward off harmful pathogens for both her and the developing fetus. Immune system changes in pregnancy are complex, including reductions in the quantity and function of NK cells and T-cells and increases in monocytes, granulocytes, and dendritic cells in the peripheral blood. Th2 cells enhance B lymphocyte activation and antibody production while concurrently suppressing the response of CTLs, resulting in a weakened cell-mediated immunity.

The development of liver damage resulting from HEV infection in the general population is primarily associated with the activation of immune cells, specifically $CD8^+$ T-cells and NK cells, both within the liver and in the peripheral circulation. In pregnant patients experiencing fulminant hepatic failure due to HEV, the presence of a 'Th2 bias' has been documented, although its exact influence on the severity of the illness remains uncertain[84].

During pregnancy, specific hormones such as progesterone, estrogen, and human chorionic gonadotropin experience significant changes in their levels. A study found that these hormonal levels were higher in pregnant patients with acute liver failure who tested positive for HEV compared to those who tested negative[85]. Notably, the increased estradiol levels in the bloodstream of HEV-infected pregnant women promote the virus's multiplication[86-88]. Moreover, high estrogen levels are linked to premature birth, underweight newborns, and fetal death due to impaired placental functioning in HEV-infected pregnant women[86-88]. Both progesterone and estrogen were found to disturb the balance between Th1 and Th2 responses[12]. Moreover, estrogen plays a direct role in reducing the cytotoxicity of $CD8^+$ T-cells [89]. It can adjust the survival and activation of B cells and can hinder the production of B cells during pregnancy[89].

Differences in maternal illness and death rates vary across HEV genotypes. The most prevalent genotypes associated with these issues in pregnancy are HEV-1 and HEV-2. However, this correlation between increased incidence and severity of hepatitis E in pregnant women exists with HEV-1 or HEV-2 but not HEV-3 and HEV-4[90-95]. In a recent study using a model based on maternal and fetal organ culture, HEV-1 exhibited more effective replication, produced more infectious particles, and induced more severe tissue changes than HEV-3[96]. This suggests that genotype might significantly impact HEV infection severity, especially in pregnant women. However, due to a lack of appropriate *in vivo* and *in vitro* models and difficulties cultivating the virus *in vitro*, a comprehensive study investigating susceptibility, infectivity, replication capacity, and pathogenicity across different HEV genotypes has not been conducted. Additionally, genetic variations in HEV can affect viral shape, development, clinical outcomes, and antiviral resistance[3,97,98]. However, it remains uncertain if these variations are linked to maternal illness or death, warranting further research.

HEV infection in immunosuppressed patients

The damaging effects of HEV infection can be amplified due to the increased prevalence of disease-related or treatment-induced immunosuppression in contemporary healthcare (Figure 4).

Research by Pischke *et al*[99] revealed that transplant recipients and immunosuppressed patients, after exposure to HEV in blood products, showed symptoms of liver disease within the standard incubation period of 50–60 d[99]. These patients are at risk of chronic HEV infection, typically defined as the ongoing detection of HEV RNA in blood and/or feces for over 3–6 months[100].

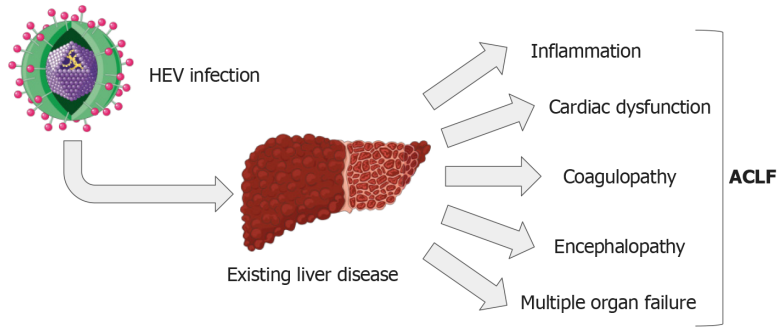

Figure 2 Overview of the impacts of acute-on-chronic liver failure resulting from hepatitis E virus infection in the context of pre-existing liver disease. HEV: Hepatitis E virus; ACLF: Acute-on-chronic liver failure. Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/Licenses/by/3.0/).

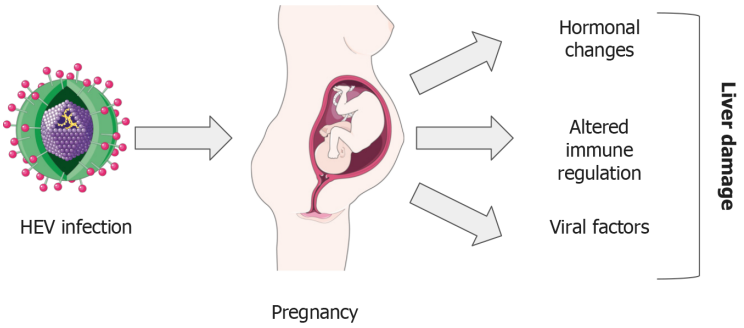

Figure 3 Summary of the adverse effects of hepatitis E infection during pregnancy. HEV: Hepatitis E virus. Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/Licenses/by/3.0/).

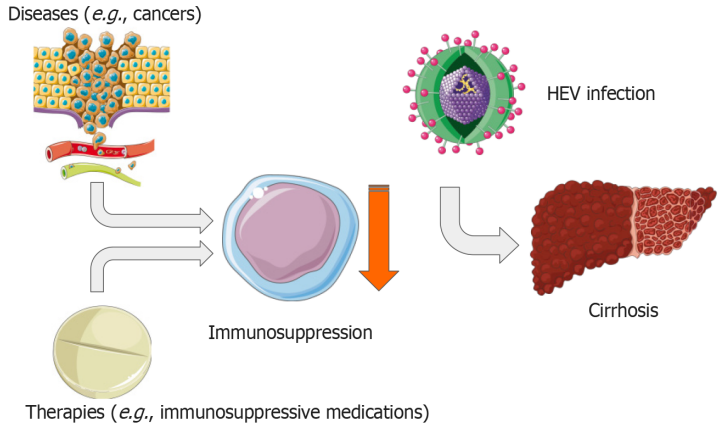

Figure 4 The effects of hepatitis E superinfection on liver health related to disease and therapeutic immunosuppression. HEV: Hepatitis E virus. Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/Licenses/by/3.0/).

The presence of long-term HEV infection was first observed in European transplant recipients in 2008[101-103]. Initially, 14 cases of acute HEV infection were reported among liver or kidney transplant recipients[104]. From these, eight developed persistent HEV infections marked by high alanine aminotransferase levels, inflammation in the portal vein, minor necrosis, and evidence of fibrosis within a year post-infection.

Nevertheless, the exact prevalence of lingering acute HEV infection among transplant recipients remains disputed. According to a retrospective, multicenter European study, it was found that 66% of transplant recipients suffering from symptomatic acute HEV infection later developed chronic hepatitis E[105]. However, the study's retrospective nature suggests possible undetected asymptomatic HEV infections. Previous studies accounting for both symptomatic and asymptomatic HEV infections among transplant recipients suggest progression to chronicity ranges from 21%–50%[106-108].

The chance of transplant recipients developing a long-term HEV infection can vary based on the specific immunosuppressive treatment and the degree of immunosuppression[73]. The use of Tacrolimus has been associated with a higher risk of chronic hepatitis compared to Cyclosporine A[105]. In contrast, a lower risk of chronic hepatitis was observed with the use of Mycophenolic acid[107]. Interestingly, laboratory tests reveal that calcineurin inhibitors could potentially increase HEV replication, while Mycophenolic acid seems to inhibit it[109]. A large-scale study involving Japanese liver transplant recipients noted a very low prevalence of chronic HEV infection, suggesting possible variations in HEV subtypes, strains, or host genetic factors influencing the persistence of the virus in the body[110,111]. While no instances of fulminant liver failure linked to HEV infection in transplant patients have been reported, there is a risk of ongoing infection and chronic hepatitis. Chronic HEV infections are mostly seen with genotype 3, with a few recorded cases involving genotype 4[112,113]. Recently, persistent HEV infections from genotypes 7 and 8, originating from camels, were identified in transplant recipients[114]. To date, no chronic HEV infections have been reported resulting from genotypes 1 or 2.

VIRAL FACTORS AFFECTING LIVER FAILURE

Despite the unclear roles of viral proteins in HEV infection and pathogenesis, intriguing evidence suggests that the genotype and specific mutations in the ORF1 region could affect factors such as the virus's replicative potential, viremia levels, immune response characteristics, cell damage degree, and disease progression.

It has been reported that genotype 1 HEV shows more efficient replication, causing a greater cytopathic effect and higher levels of IL-6, CCL-3, CCL-4, and CXCL10 than genotype 3. This difference was observed in lab-cultured tissue explants from the decidua basalis and placenta[96]. Genotype 1 reduced the production of type III IFNs, while genotype 3 had no impact. The impact of more efficient replication by genotype 1 HEV might explain the severe progression of HEV infection during pregnancy[96].

In addition, the V239A mutation in the helicase produced by ORF1 has been associated with acute hepatitis cases, potentially suggesting greater virulence of genotype 3[115]. Similarly, 11 mutations in various ORF1 regions that seem to affect HEV infection severity have been identified. These include V27A, D29N, H105R, F179S, A317T, T735I, L1110F, V1120I, F1439, C1483, and N1530T[116-119].

Particularly, the D29N and V27A mutations in the ORF1-encoded methyltransferase are strongly linked with adverse outcomes in acute liver failure cases[117]. Genotype 1 HEV strains carrying the A317T and V1120I mutations–found in the Y and helicase regions of ORF1, respectively–exhibit enhanced replicative potential and are frequently seen in patients experiencing fulminant hepatic failure (Figure 5)[119].

ORF1 encodes multiple proteins responsible for managing the post-translational modification (PTM) of cellular proteins, which impacts protein stability and function. Notably, these proteins can influence deconjugation of a variety of substrates, such as a specific fluorescent probe (7-amino-4-methylcoumarin)-labeled ubiquitin, ISG15, Nedd8, and Small Ubiquitin-like Modifier (SUMO)[19]. Although the HEV methyltransferase-PCP displayed marginally impactful deneddylation and deSUMOylation effects, its deubiquitination and deISGylation activities were potent, potentially severely hampering the antiviral effects of IFNs[19].

Separate studies have revealed that the Macro domain protein, encoded by ORF1, interferes with cellular ADP-ribosylation[23]. There are two known forms of ADP-ribosylation: Poly (ADP-ribosyl) ation and mono (ADP-ribosyl) ation, commonly referred to as PARylation and MARylation, respectively[120,121]. These modifications regulate a myriad of cellular processes, including DNA damage response, unfolded protein response, cell cycle progression, cell death, metabolism, and immune responses[122-125]. Enzymes in both mammals and microbes can attach, erase, or recognize ADP-ribosyl moieties of target proteins[126].

The Macro domain protein in HEV operates as an ADP-ribose-protein hydrolase that removes mono-ADP-ribose and/or poly (ADP-ribose) modifications from target proteins, processes known as deMARylation and dePARylation, respectively[23]. Given the extensive effects of ADP-ribosylation, it is hypothesized that the Macro domain protein is a key player in the way HEV neutralizes the antiviral effect of IFNs and disrupts immune responses (Figure 5)[23].

These findings present a potential new therapeutic target, and inhibitors of the HEV Macro domain protein are deemed promising treatments for severe hepatitis E4[127-129]. Furthermore, PTMs have been associated with the disease mechanisms of several conditions, including alcohol-induced liver injury, MASLD/MASH, and cirrhosis[128-133]. Additional research is needed to explore how HEV infection influences the abnormal PTM patterns linked with preexisting liver diseases and its potential effects on disease progression.

The capsid protein produced by ORF2 can interfere with IFN activation by preventing IRF-3 phosphorylation. This obstruction stems from its interaction with certain proteins, including MAVS, TANK-binding kinase 1, and IRF3 (Figure 5)[134]. It has recently been discovered that ORF2 encodes a unique protein that undergoes glycosylation and can be released as a dimer into either the patient's bloodstream or the supernatants of HEV-infected cell cultures[135]. The proteins that ORF2 expresses may interfere with or evade the host's innate immunity. Furthermore, research suggests the capsid protein aids in HEV's survival or replication within infected hepatocytes. For example, it prevents cellular NF-κB activity in human liver cancer cells by obstructing the ubiquitin-mediated proteasomal degradation of IκBα, potentially enhancing the survival of HEV-infected hepatocytes (Figure 5)[136].

The protein produced by ORF3 is believed to cause coagulopathy linked with HEV, disturbing the balance of the coagulation and fibrinolysis processes[137]. In addition, the ORF3 protein has a role in controlling carbohydrate metabolism and mitochondrial function[138]. Importantly, it also contributes to the disruption of immune response. This HEV protein downregulates the expression of certain cytokine genes, such as IFNs, by reducing the levels of TLR3 and

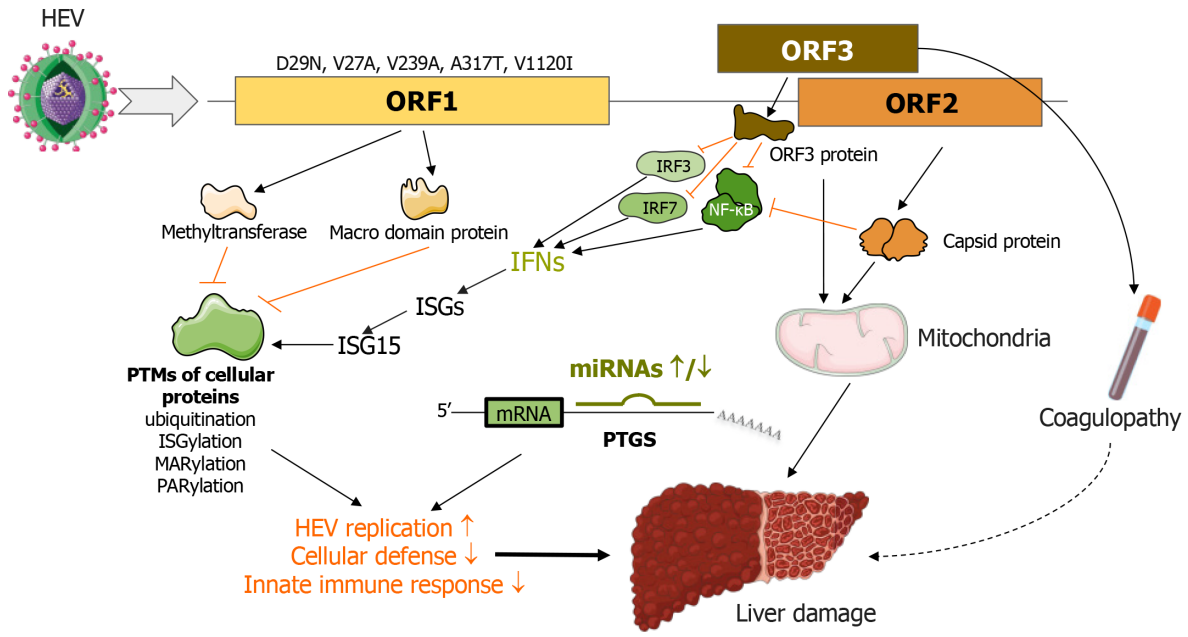

Figure 5 Several viral factors that increase hepatic injury induced by hepatitis E virus. The solid line with black arrows represents a direct influence, whereas the dashed line with arrows represents an indirect impact. Red lines show inhibitory processes. D29N, V27A, V239A mutations are associated with increased virulence. HEV: Hepatitis E virus; ISGs: Interferon-stimulated genes; IRF: Interferon regulatory factor; miRNA: MicroRNA; NF-κB: Nuclear factor-κB; ORF: Open reading frame; PTM: Post-translational modification; PTGS: Post-transcriptional gene silencing. Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/Licenses/by/3.0/).

TLR7 and the activation of NF-κB, IRF3, and IRF7[139]. It also inhibits STAT-1 phosphorylation, which dampens IFN signaling and antiviral ISG induction (Figure 5)[54,55].

Cellular miRNAs are non-coding RNA molecules that regulate gene expression by inducing post-transcriptional gene silencing. With a broad impact range, miRNAs are crucial regulators in many physiological processes and pathological conditions. They are involved in liver regeneration, metabolism, fibrosis, inflammation, and carcinogenesis, with contributions to the pathogenesis of diseases such as cirrhosis, ACLF, MASLD/MASH, and liver cancer[140-143].

Several viruses, including HEV, are known to encode miRNAs[53]. To illustrate, HEV-miR-A6 aids viral replication and deters the host's innate immune response by inhibiting the expression of type I IFNs[53]. Additionally, the cellular miR-122 could boost HEV replication[144].

Studies on pregnant women's PBMCs reveal that HEV infection alters cellular miRNAs' expression patterns. They identified miR-450b as a significant indicator of mortality in acute liver failure amongst HEV-infected pregnant women [145]. Consistent with this, miRNA profiling shows that miRNAs circulating in HEV-infected patients' plasma could indicate self-resolving acute and chronic hepatitis E, as well as acute liver failure[146,147].

Furthermore, miRNA signatures identified in patients' sera could link with hepatitis E-specific immune dysfunction [146,148]. It is noted that miRNAs can be packed in exosomes, which HEV virion particles use for egress[44]. Comparing the miRNA profiles of exosomes from healthy and HEV-infected blood donors, it was evident that HEV infection significantly alters the miRNA content of exosomes[149].

These fascinating studies have stirred interest in the potential use of miRNAs-targeting compounds as therapeutic options for severe hepatitis E.

These viral proteins play complex roles in HEV infection and disease development, highlighting the need for more research to fully understand their functions and possible treatment targets. More studies in this field could help develop effective methods for managing HEV-related liver failure and other complications. A deeper understanding of the virus also encourages investigations into new treatment targets, like miRNAs and enzymes involved in PTMs, thus expanding the range of potential drug targets.

CONCLUSION

Cirrhosis is associated with profound liver, intestinal and immune dysfunction. In patients with cirrhosis, the composition of the intestinal microbiota changes significantly, the ratio of commensal bacteria decreases, whereas the proportion of pathogenic species increases. The intestinal barrier function is damaged, leaky gut syndrome develops, and the continuous flow of intestinal bacteria and their various products into the bloodstream leads to persistent stimulation of the immune system. As a result, a profound functional impairment develops in the innate and adaptive immunity. HEV is a common cause of acute hepatitis, in healthy individuals, the infection is mostly asymptomatic or associated with minor symptoms. However, HEV can establish a chronic infection in immunosuppressed individuals. The most

surprising and frightening feature of hepatitis E is that it can lead to acute decompensation of cirrhosis, and fulminant hepatic failure with a high mortality rate in pregnant women. Interesting observations suggest that the joint effects of immunopathological processes are responsible for the severe course of hepatitis E among at-risk individuals. The discovery of unknown elements of the HEV-specific immune response may facilitate the development of more efficient monitoring and therapeutic strategies.

FOOTNOTES

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Country/Territory of origin: Hungary

ORCID number: László Orosz 0000-0002-5268-6134; Károly Péter Sárvári 0000-0001-9926-0918; Áron Dernovics 0000-0003-3759-2524; András Rosztóczy 0000-0002-8597-8934; Klára Megyeri 0000-0002-8255-8272.

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REVIEW

Harnessing immunity: Immunomodulatory therapies in COVID-19

Tsvetelina Velikova, Hristo Valkov, Anita Aleksandrova, Monika Peshevska-Sekulovska, Metodija Sekulovski, Russka Shumnalieva

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Tsvetelina Velikova, Monika Peshevska-Sekulovska, Metodija Sekulovski, Russka Shumnalieva, Medical Faculty, Sofia University St. Kliment Ohridski, Sofia 1407, Bulgaria

Hristo Valkov, Department of Gastroenterology, University Hospital "Tsaritsa Yoanna-ISUL", Medical University of Sofia, Sofia 1527, Bulgaria

Anita Aleksandrova, Department of Immunology, SMDL Ramus, Simitli 6000, Bulgaria

Monika Peshevska-Sekulovska, Department of Gastroenterology, University Hospital Lozenetz, Sofia 1407, Bulgaria

Metodija Sekulovski, Department of Anesthesiology and Intensive Care, University Hospital Lozenetz, Sofia 1407, Bulgaria

Russka Shumnalieva, Department of Rheumatology, Clinic of Rheumatology, University Hospital "St. Ivan Rilski", Medical University-Sofia, Sofia 1612, Bulgaria

Corresponding author: Russka Shumnalieva, MD, PhD, Adjunct Associate Professor, Department of Rheumatology, Clinic of Rheumatology, University Hospital "St. Ivan Rilski", Medical University-Sofia, Urvich Street 13, Sofia 1612, Bulgaria. rshumnalieva@yahoo.com

Abstract

An overly exuberant immune response, characterized by a cytokine storm and uncontrolled inflammation, has been identified as a significant driver of severe coronavirus disease 2019 (COVID-19) cases. Consequently, deciphering the intricacies of immune dysregulation in COVID-19 is imperative to identify specific targets for intervention and modulation. With these delicate dynamics in mind, immunomodulatory therapies have emerged as a promising avenue for mitigating the challenges posed by COVID-19. Precision in manipulating immune pathways presents an opportunity to alter the host response, optimizing antiviral defenses while curbing deleterious inflammation. This review article comprehensively analyzes immunomodulatory interventions in managing COVID-19. We explore diverse approaches to mitigating the hyperactive immune response and its impact, from corticosteroids and non-steroidal drugs to targeted biologics, including anti-viral drugs, cytokine inhibitors, JAK inhibitors, convalescent plasma, monoclonal antibodies (mAbs) to severe acute respiratory syndrome coronavirus 2, cell-based therapies (*i.e.*, CAR T, *etc.*). By summarizing the current evidence, we aim to provide a clear roadmap for clinicians and researchers navigating the complex landscape of immunomodulation in COVID-19 treatment.

Key Words: Immunomodulation; COVID-19; SARS-CoV-2; Immunotherapy; Antiviral immune response; Cytokine storm; Adaptive immunity; Therapeutic strategies; Immune modulators; Viral infection; Host immune response

Core Tip: Effective management of coronavirus disease 2019 (COVID-19) requires a nuanced approach that harnesses the host's immune response Immunomodulatory therapies play a pivotal role in fine-tuning the immune system, striking a balance between defense and avoiding excessive inflammation. In line with this, increased precision in targeting specific immune pathways, alongside personalized treatment strategies, holds promise in optimizing outcomes for COVID-19 patients. This paper explores the evolving landscape of immunomodulation, emphasizing its potential as a crucial component in the therapeutic arsenal against the virus.

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INTRODUCTION

In late 2019, the world was overrun by a severe respiratory virus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1]. Since the population was non-immune and the virus spread quickly from person to person, global healthcare met an immense burden. Symptoms characteristic of the coronavirus including fever, shortness of breath, fatigue, as well as major complications like pneumonia, sepsis, respiratory distress and septic shock led to the use of various types of symptomatic therapies before the development of coronavirus disease 2019 (COVID-19) vaccines[2].

Patients are treated in accordance with the degree of illness, as a specialist supervises mandatory treatment. Oxygen therapy is administered if the degree of illness is severe (such as in the case of pneumonia). Immunosuppressors are suitable for treatment, as they can disrupt the induction of interferons type I. This shows why the use of proper immunomodulators could be utilized.

Immunomodulators are medically necessary because the exact duration of the protection provided by antibodies against COVID-19, which the vaccine and infection produce, has not yet been established. Several immunomodulators have been administered to patients: immunotherapy with interferon, mAbs, anti-inflammatory cytokine, plasma, prebiotics, probiotics, stem cells, vitamins, *etc*.

Infection with COVID-19 induces a destructive immune hyperreaction through several pathways. One of these is Toll-like receptor (TLR) activation, which leads to a cytokine storm characterized by overproduction of inflammatory factors and cytokines[3].

This cytokine storm is the underlying cause of viral sepsis and inflammation-induced lung damage, pneumonitis, acute respiratory distress syndrome (ARDS), respiratory failure, shock, organ failure and death. Therefore, it is essential to develop strategies that can counteract or neutralize this cytokine storm by modulating the immune response and thereby suppressing lung inflammation and injury, inhibiting viral replication, and promoting viral clearance, thus ultimately saving lives[4].

The global trajectory of the COVID-19 pandemic has underscored the importance of understanding and effectively modulating the intricate dynamics of the host immune response. This viral infection, caused by the novel SARS-CoV-2, manifests as a spectrum of clinical outcomes, ranging from mild respiratory symptoms to severe pneumonia and life-threatening complications. As researchers and clinicians grapple with the evolving nature of this virus, the central role of immunity has become increasingly evident. However, this recognition has brought to light a dual challenge: the need to achieve a delicate balance between an insufficient immune response that may foster viral persistence and an exaggerated response that can lead to detrimental hyperinflammation[5].

The inadequacy of the initial immune response poses a formidable obstacle in the battle against COVID-19. SARS-CoV-2, with its ability to evade immediate immune detection, gains a foothold in the respiratory system, often resulting in prolonged viral shedding and increased transmission. This delayed immune activation facilitates viral spread and allows the virus to establish a stronghold, particularly in susceptible individuals. For that reason, understanding the factors contributing to this delayed immune response is crucial for devising effective therapeutic strategies[6].

Conversely, an overly exuberant immune response, characterized by a cytokine storm and uncontrolled inflammation, has been identified as a significant driver of severe COVID-19 cases. The immune system's attempt to eliminate the virus becomes a double-edged sword, inadvertently causing collateral damage to host tissues. This hyperinflammatory state, if left unchecked, can lead to ARDS, multi-organ failure, and an increased risk of mortality. Consequently, deciphering the intricacies of immune dysregulation in COVID-19 is imperative to identify specific targets for intervention and modulation[7].

With these delicate dynamics in mind, immunomodulatory therapies have emerged as a promising avenue for mitigating the challenges posed by COVID-19. Precision in manipulating immune pathways presents an opportunity to alter the host response, optimizing antiviral defenses while curbing deleterious inflammation[8].

As the COVID-19 pandemic persists, the intricacies of the host immune response have emerged as central players in the battle against the virus. However, this nuanced interplay presents a dual challenge—on the one hand, an inadequate immune response may result in viral persistence. On the other hand, an overly exuberant reaction can lead to detrimental hyperinflammation. Navigating this delicate balance demands a comprehensive understanding of immunomodulation strategies[9].

This paper critically examines the multifaceted challenges inherent in the immunological landscape of COVID-19, emphasizing the imperative for targeted immunomodulatory interventions to navigate the fragile equilibrium between adequate viral clearance and immune-mediated pathology. By unraveling the complexities of host-virus interactions, we aim to contribute to the evolving strategies to harness immunity for optimal outcomes in the fight against COVID-19.

PATIENTS WITH AUTOIMMUNE DISEASES AND COVID-19: LESSONS FOR IMMUNOMODULATION THERAPY FOR COVID-19

The infection with SARS-CoV-2 is followed by a hyperactive inflammatory response, which is crucial for the pathogenesis of COVID-19. Among the known risk factors for a more severe clinical course of COVID-19, including risk of hospitalization and death, are older age, and co-morbidities like diabetes, and cardiovascular or chronic kidney diseases, which are even more prominent risk factors in immunocompromised patients. The latter include patients with rheumatic diseases, hematological malignancies, organ transplants, HIV or other immunodeficiency disorders for which a prolonged shedding of SARS-CoV-2 RNA has been reported[5,9].

Corticosteroids (CS) have anti-inflammatory effects against a broad spectrum of cytokines and chemokines. They could suppress lung injury and multisystem organ dysfunction during hyperinflammatory stages in patients with severe or critical COVID-19 disease who require oxygen therapy. The beneficial effects of the use of CS in hospitalized patients have been proven in multiple randomized trials, which showed that the introduction of dexamethasone in the treatment protocol leads to a reduction of the mortality rate in the group of patients who required invasive mechanical ventilation or non-invasive oxygen support (RECOVERY trial, CoDEX trial, *etc.*)[10]. Current recommendations are against the use of dexamethasone or other systemic corticosteroid in non-hospitalized patients without other indications. Although inhaled CS suppress the inflammation in the lungs by impairing viral replication and downregulating receptor expression, currently, there is insufficient evidence for or against their use[10-13].

Studies have shown that during SARS-CoV-2 infection, the bronchial epithelial cells produce high amounts of interleukin 6 (IL-6) in a dose-dependent manner. An activated IL-6-JAK-STAT3 axis characterizes severe COVID-19 disease, and levels of IL-6 are associated with the severity of COVID-19, independent of age and sex. IL-6 inhibitors such as tocilizumab and sarilumab have been evaluated in clinical trials for treating hospitalized patients with systemic hyperinflammatory status. Currently, tocilizumab is approved for treating hypoxic patients on systemic CS[14,15].

JAK inhibitors were also discussed for COVID-19 treatment. Activation of JAK molecules by proinflammatory cytokines such as IL-6 leads to phosphorylation of STAT proteins followed by immune activation and inflammation. Thus, JAK inhibitors are studied for the treatment of COVID-19 disease. Currently, only baricitinib is approved by the US Food and Drug Administration (FDA) for the treatment of hospitalized hypoxic COVID-19 patients[16].

Early on in the pandemic, antimalarials were used as inhibitors of cytokines production and viral fusion. Systemic literature review of hydroxychloroquine (HCQ) and chloroquine (CQ) with azithromycin (AZ) did not prove any benefit. Currently, antimalarials are not recommended for treatment of hospitalized patients with COVID-19[17,18].

The impaired immune system in patients with autoimmune rheumatic diseases (AIRDs), along with the effects of CS and disease-modifying antirheumatic drugs, which further suppress the immune system, increase the risk of infections compared to the general population. Being immunosuppressed, even vaccinated, patients with AIRDs are at higher risk for COVID-19 and severe COVID-19. This risk is reported to be related also to the ongoing treatment for the underlying rheumatic condition. Thus, in using immunosuppressive agents when AIRDs, the clinician should balance the benefit-risk ratio considering the two aspects of the immunosuppressive treatment[19]. As these medications can modulate different aspects of the immune response, they could be used as monotherapy or in combination in clinical practice to reduce the severity of COVID-19 course. On the other hand, immunosuppressive agents could reduce the suppressive immune response to viral replication, thus prolonging the viral survival, infection and shedding. CS are widely used to induce remission or as bridging therapy for the long-term management of patients with AIRDs. The beneficial effect of CS in managing patients with AIRDs is related to their rapid inhibition of the immune cell response. By inhibiting the host-immune response, they potentiate a delay in viral clearance and, in case of a SARS-CoV-2 infection, could increase the risk of lung involvement. Oral CS are reported to have a negative impact on AIRD patients with COVID-19 disease, with doses over 10 mg/prednisolone equivalent daily related to increased risk of hospitalization[19]. Moreover, the dose of CS is reported to be an independent risk factor for COVID-19-related death[20]. However, glucocorticoids have side effects that include diabetes mellitus and hypertension, and should be avoid when applicable.

Data reports on clinical outcomes in patients with AIRD treated with agents suppressing T-cells (*e.g.*, calcineurin inhibitors, mycophenolate mofetil), B-cells (anti-CD20 antibodies, anti-CD22 antibodies) or agents against type I interferon show that this treatment agents lead to more severe COVID-19 disease[9,21,22].

Treatment with rituximab has been reported to increase the risk of severe COVID-19 and to lead to the poorest outcome in different studies[23,24]. One suggested mechanism is due to the low viral clearance and persisting viremia[25, 26]. Rituximab impairs B cell response to the COVID-19 vaccine in patients with AIRD, thus making the treatment with Rituximab not preferable even in vaccinated individuals[27,28].

Data on the use of anti-TNFα agents in AIRDs infected with COVID-19 show the relative safety of these agents with lower risk of hospitalization and better clinical outcomes compared to patients treated with other immunosuppressants. A possible mechanism is the blockage of TNF-α cytokine as one of the contributing factors in the "cytokine storm" related to COVID-19[29,30].

Other immunosuppressants such as azathioprine, cyclophosphamide, ciclosporin, mycophenolate or tacrolimus were reported to be associated with a higher risk of COVID-19-related death compared to patients on methotrexate monotherapy. The same was valid for patients with AIRDs not receiving any disease-modifying antirheumatic drug (DMARD)[31,32].

IMMUNOMODULATION MODALITIES FOR COVID-19

SARS-CoV-2 possesses many mechanisms to blunt early immune responses, allowing viral replication and worsening clinical symptoms and, in some cases, uncontrolled immune activation (*i.e.*, cytokine storm)[33].

While the exact pathways causing cytokine release syndrome (CRS) and ARDS are still unknown, high levels of proinflammatory cytokines like IL-6, IL-1β, and TNF-α characterize the cytokine storm. There are encouraging preliminary data in CRS and ARDS with immunomodulators like Tocilizumab, an IL-6 inhibitor; these agents may be used alone or in conjunction with other treatments, such as dexamethasone, in severe disease. One important mechanism contributing to COVID-19-associated mortality may be the cytokine storm augmenting lung injury[33]. Larger studies and randomized controlled trials are necessary to validate the majority of the evidence currently available on therapeutic agents, which is based on small observational studies. Because therapeutic agents and their administration are poorly understood, studies evaluating all aspects of therapy—including the timing of administration, potential synergism between treatments, and potential toxicities—are desperately needed. Additionally, it is critical to strike a balance between the need to maximize patient safety and expedite the utilization of potentially helpful medications.

Iqbal Yatoo *et al*[34] focused on immunomodulatory therapies available before COVID-19 vaccines or specific treatment: convalescent plasma, immunoglobulins, monoclonal or polyclonal antibodies, immunomodulatory agents, cell-based therapies (*i.e.*, NK cells, T cells, stem cells), cytokines and toll-like receptors based therapies.

EMERGING ANTI-VIRAL THERAPEUTIC OPTIONS

Viral mRNA synthesis inhibitors

Remdesivir (GS-5734) was the only medication for the treatment of COVID-19 with emergency use authorization issued by the FDA[35]. Incorporating into the viral RNA as a nucleotide/adenosine analog, Remdesivir results in premature termination of the viral replication[36-38]. In human airway epithelial cells, remdesivir inhibits Middle East respiratory syndrome coronavirus and SARS-CoV[39]. Nausea, vomiting, elevation of transaminases, and diarrhea are some adverse effects of remdesivir use[35]. At ten hospitals in Hubei, China, a randomized, double-blind, placebo-controlled, multicentre trial (NCT04257656) was conducted to evaluate the safety and efficacy of Remdesivir in hospitalized adult patients with severe COVID-19[40]. Two hundred thirty-seven patients were assigned to a 2:1 ratio (158 to Remdesivir and 79 to placebo), and the participants were permitted concomitant use of interferons, CS, and lopinavir-ritonavir. Despite the adequate tolerability, the study did not find statistically significant clinical benefits of Remdesivir use[40]. In a double-blind, randomized, placebo-controlled trial, Beigel *et al*[41] concluded that intravenous Remdesivir is superior to a placebo in shortening the time to recovery in hospitalized adult patients with COVID-19, who had evidence of lower respiratory tract infection.

Favipiravir, known as T-705, is an antiviral drug that selectively and potently inhibits the RNA-dependent RNA polymerase[42] and may serve as an emerging treatment for COVID-19[35]. Tocilizumab represents a recombinant humanized monoclonal antibody against the human IL-6 receptor[43,44]. Multicenter, randomized controlled trial enrolled 26 patients to assess the efficacy and safety of tocilizumab combined with favipiravir in patients with COVID-19. The participants were divided into three groups according to treatment: favipiravir, tocilizumab, or tocilizumab combined with tocilizumab. The study showed the beneficial effect of tocilizumab on COVID-19 patients, as tocilizumab alone or combined with favipiravir can improve pulmonary inflammation and reduce mortality and worsening of the infection[44]. Generally, Favipiravir is well tolerated, however, liver dysfunction, diarrhea, and nausea are some of the side effects related to its use[45]. Resulting in the inhibition of membrane fusion between virus particles and plasma membranes related to its intercalation into membrane lipids[46], umifenovir is another candidate drug for the treatment of COVID-19[35]. Chen *et al*[47], comparing favipiravir with umifenovir, did not find a significant difference in the clinical recovery rate at day 7 in adult patients with COVID-19.

CQ and its derivative, HCQ, are antimalarial drugs that showed activity against numerous RNA viruses and were considered as promising therapeutic options for COVID-19[35,48,49]. The limitation of the virus-cell fusion, the causing of alkalization of the usually acidic endosomal pH of the infected cells, and receptors modifying glycosylation are some of the multiple anti-viral mechanisms of CQ and HCQ[35,50]. The current evidence shows that HCQ is inefficient in

reducing mortality and has no benefits regarding time to clinical improvement and intensive mechanical ventilation requirements[48]. Common adverse reactions related to treatment with CQ and HCQ are diarrhea, abdominal discomfort, nausea, and vomiting[51]. Cardiac toxicity and prolonged QTc interval are also described with these drugs, and retinopathy is the most severe complication of CQ/HCQ[51]. The HCQ and AZ combination showed a synergetic effect on SARS-CoV-2-infected cells in in vitro studies[52]. AZ is a macrolide associated with a higher risk of cardiac death and prolongation of the QT/QTc interval[53,54]. Based on the current evidence, HCQ, either alone or with AZ, is unsuitable for treating COVID-19[55].

Geldanamycin represents the group of ansamycins and inhibits the HSP90[56]. Anticancer and antimicrobial properties characterize Geldanamycin and demonstrate antiviral activities against viruses such as HIV-1 and Influenza[56]. Hepatotoxicity and anemia are some of the adverse effects of the use of Geldanamycin, and there is no registered trial of the impact of this medication on patients with COVID-19[35].

Thalidomide has anti-inflammatory activity, downregulates soluble levels of mediators such as TNF- α, IL-1, IL-6, and PGE2, and inhibits the COX-2[57]. The FDA approved Thalidomide for treating multiple myeloma and erythema nodosum leprosum[58]. Through its immunomodulatory and anti-inflammatory properties, Thalidomide could be tested in treating respiratory complications related to COVID-19[58]. It is a well-known fact that Thalidomide has a teratogenic effect[59].

Non-steroidal anti-inflammatory drugs (NSAIDs) are available over the counter (OTC) in most countries, and according to the available evidence, NSAIDs neither worsen outcomes of COVID-19 nor increase the likelihood of SARS-CoV-2 infection and could be used as antipyretic and analgetic drugs during COVID-19[60].

Losartan is a selective antagonist of the AT1 receptor that potentially can protect against lung damage induced by COVID-19 by inhibiting the ACE–Ang II–AT1 axis, which is implicated in fibrosis[61]. In an individual participant data meta-analysis including 325 participants, Di Stefano *et al*[62] did not find a benefit of losartan versus control treatment in hospitalized patients with COVID-19.

IL-1 INHIBITORS

The IL-1 family consists of 11 members as IL-1α and IL-1β are proinflammatory proteins that share IL-1 receptor 1 as their common receptor[63]. Anakinra is a recombinant human IL-1 receptor antagonist[64]. Some therapeutic indications of anakinra are Still's Disease, Rheumatoid Arthritis, and Cryopyrin-Associated Periodic Syndromes (European Medicines Agency Summary of product characteristics)[65]. In a systematic review and meta-analysis published in 2023, Dahms *et al* [66] concluded that compared to placebo or standard care alone, Anakinra shows no effectiveness on adult hospitalized patients with SARS-CoV-2 infection regarding mortality and clinical improvement.

Serum IL-6 is significantly elevated in patients with complicated COVID-19, and increased IL-6 predicts adverse clinical outcomes[67]. Tocilizumab represents a recombinant humanized, anti-human monoclonal antibody against membrane-bound and soluble interleukin 6 receptors (IL-6R)[42]. In a systematic review and meta-analysis, Keske *et al* [68] showed the effectiveness of tocilizumab in non-intubated cases with severe COVID-19. Through this systematic review and meta-analysis, they demonstrated that compared to standard-of-care (SOC) treatment tocilizumab decreased the need for invasive mechanical ventilation (OR: 0.76; 95%CI: 0.67–0.86, $P < 0.001$ and for the heterogeneity $I^2 = 6\%$, $P = 0.39$) and reduced the decreased the mortality (OR: 0.84; 95%CI: 0.73–0.96, $P = 0.009$, and for the heterogeneity $I^2 = 0\%$, $P = 0.82$)[68].

Sarilumab is a human recombinant IgG1 monoclonal antibody that inhibits IL-6-mediated signaling by binding to both soluble and membrane-bound IL-6R[69]. In the treatment of severe COVID-19, Sarilumab was considered as an alternative to tocilizumab[70]. In a national, multicenter, open-labeled, phase 3 randomized clinical trial, Mastrorosa *et al* [71] evaluated the clinical efficacy and safety of intravenous sarilumab in addition to the SOC in managing adults with severe COVID-19 pneumonia. The participants in the trial were randomly assigned in a 2:1 ratio to receive sarilumab in addition to SOC or SOC therapy alone[71]. Mastrorosa *et al*[71] did not find the efficacy of adding sarilumab in severe COVID-19. Increased risk of secondary infections, hypotension, cytopenias, and edemas are some of the side effects related to sarilumab use[35].

JAK INHIBITORS

Via receptor-mediated endocytosis, SARS-CoV-2 enters cells after binding its spike protein to the human ACE-2 receptor [72]. AAK1 is one regulator of endocytosis whose inhibition could prevent SARS-CoV-2 entry and the intracellular assembly of virus particles[73]. Cycling GAK modulates endocytosis[36]. By binding to GAK and inhibiting the AAKI kinase, it is hypothesized that baricitinib prevented viral infection[74], and by targeting JAK1 and JAK2, baricitinib would inhibit inflammation[75]. Baricitinib is recommended as the therapeutic option for patients with severe COVID-19[70]. In a systematic review and meta-analysis, Song *et al*[76] showed that compared to the standard treatment, baricitinib decreases mortality and mechanical ventilation requirements in patients with severe COVID-19.

Tofacitinib is an oral, small molecule JAKi[77], which inhibits JAK1 and JAK 3 and, to a lower degree, JAK 2, modulating on this way the JAK-STAT signaling[78,79]. Tofacitinib can decrease the release of cytokines by type 1 and type 17 helper T cells by modulating the action of IL-6 and interferons[80-82]. In the STOP-COVID study, a multicenter, randomized, double-blind, placebo-controlled trial, Guimarães *et al*[83] evaluated the efficacy and safety of tofacitinib in hospitalized patients with COVID-19 pneumonia. The trial showed that respiratory failure or death through day 28

occurred in 29.0% of those in the placebo group in 18.1% of the patients in the tofacitinib group (risk ratio: 0.63, 95%CI: 0.41 to 0.97; $P = 0.04$)[83].

CS

Glucocorticoids are among the most widely prescribed drugs with their immune-suppressive and anti-inflammatory effect[84]. The current guidelines for the treatment of COVID-19 recommend against the use of dexamethasone or other systemic CS in non-hospitalized patients in the absence of another indication[70]. The RECOVERY trial demonstrates the reduced 28-d mortality among hospitalized patients with COVID-19 using dexamethasone compared to the usual standard of care, along with other investigators, such as Ahmed and Hassan[85]. The benefit of dexamethasone was seen only among participants receiving either oxygen alone or invasive mechanical ventilation at randomization but not among those receiving no respiratory support at enrollment[85]. In a systematic review and meta-analysis, Albuquerque *et al*[86] showed that in comparison to tocilizumab, baricitinib, and sarilumab are associated with high probabilities of similar mortality reductions among hospitalized COVID-19 concurrently treated with CS.

As a result of the absence of SARS-CoV-2-specific antiviral medications, the effectiveness of COVID-19 treatments is reduced. Several COVID-19 therapies are now under investigation. However, the majority of them lack specificity, efficacy, and safety[87]. Immunotherapy is a ground-breaking medical treatment that manipulates the immune system to fight diseases. Translational research is rapidly progressing, recognized as a significant breakthrough in 2013[88]. Among the immunotherapeutic options for treating COVID-19 are Immunoglobulin, CP, antibodies, mAbs (mAbs), NK cells, T cells, TLR, cytokine therapies and immune modulators.

NSAIDS AND PARACETAMOL (ACETAMINOPHEN)

All the therapeutic options discussed above ranging from CS to targeted biologics have shown more than promising results in the battle of tackling COVID-19 infection. In patients who are hospitalized due to the severity of their condition the above-mentioned treatments are an integral part of the management plan. However, a major disadvantage in their use is that they are not readily available to the general public who are not warranted a hospital stay but are still dealing with the consequences of this viral infection.

This is where OTC drugs come into play, more specifically NSAIDs, which are a great alternative for in-home-care therapy of COVID-19[89]. NSAIDs help with pain management and fever reduction due to their analgesic and antipyretic properties respectively, which makes them suitable for symptomatic relief[90]. NSAIDs when started early enough in the disease progression have proven effective in reducing the rate of hospitalization which delivers an additional benefit for their OTC use[91].

There cannot be a critical conversation regarding the role of NSAIDs in the light of COVID-19 infection without also addressing their controversial initial speculative use at the onset of the COVID-19 pandemic when there were concerns raised about the potential for NSAIDs namely Ibuprofen to potentially heighten susceptibility for SARS-CoV-2 infection and adversely affect clinical results. These speculative claims about Ibuprofen's potential harm originated from an article in The *Lancet Respir Med*[92] in which it was stated that Ibuprofen administration runs the risk of increasing the expression of ACE-2, the receptor through which the SARS-CoV-2 virus enters cells. Increased levels of ACE2 were speculated to theoretically enhance the virus's ability to infect cells.

The confusion and unfounded fear around the topic was further exacerbated by the Health Ministry of France's endorsement of the now proven false claims[93]. Other concerns raised at the time were connected to yet another theoretical speculation of NSAIDs masking COVID-19 symptoms and thus prolonging the disease duration and therefore increasing the risk of complications[94]. All of the initial reluctance to Ibuprofen and overall NSAIDs use has been debunked and proven wrong by numerous original research articles and extensive literature reviews[95-97].

Moreover, their OTC use and lack of any harm in it is supported fully by the World Health Organization, FDA and European Medicines Agency[98].

Etoricoxib

Several characteristics, one of which is being a selective COX-2 inhibitor[99], suggest that etoricoxib could potentially suppress the cytokine storm, providing a feasible option for COVID-19 treatment. Hence, it seems justifiable to explore etoricoxib extensively to determine its suitability for repurposing as a therapeutic intervention for COVID-19[100].

Ibuprofen

While there were initial concerns about ibuprofen use in COVID-19, current evidence suggests that ibuprofen can be used for symptom management in individuals with mild to moderate illness[101]. In fact, the use of ibuprofen did not show any correlation with adverse clinical results when compared to the administration of paracetamol or abstaining from antipyretic treatment altogether[95].

Paracetamol

Paracetamol, also known as acetaminophen, is typically not classified as an NSAID due to its limited anti-inflammatory effects. Its relieving pain and fever properties, however, make it a perfect option for OTC use in COVID-19[102].

Paracetamol is generally considered safe when used at recommended doses. It has a favorable safety profile and is well-tolerated by most individuals with fewer gastrointestinal side effects compared to NSAIDs[103].

Acetylsalicylic acid (aspirin)

Aspirin causes a statistical decrease in the severity of the disease expression as well as lowers the likelihood of developing SARS-CoV-2 infection altogether[104] Meta-analyses show that Aspirin usage is associated with reduced mortality rates [105,106] while also not contributing to an increased bleeding risk, which makes it a safe option for OTC use in COVID-19 [107]. It is additionally associated with a reduced risk of embolism occurrence during the infection period[108].

Aspirin should not be stopped during hospitalization if the patient is already taking it as a part of their regular medication as this would cause worse outcomes, poorer clinical course and increased mortality compared to the alternative of keeping aspirin as part of the hospital treatment regimen[109].

In fact, patients who take Aspirin as their regular treatment for primary or secondary prevention of cardiovascular disease have a hypothesized already implemented protective benefit when it comes to COVID-19. Research proved that to be true for patients taking the drug with primary prevention focus[110].

For the geriatric population, however, such firm positive effects are not as easily observed. Explanation for this discrepancy provides the fact that the elderly population has a higher frequency of chronic comorbidities which are known to worsen COVID-19 outcomes[111].

More studies need to be conducted to completely assess Aspirin's full efficacy and clinical potential in COVID-19[112]. Nevertheless, the already established advantages of its utilization make it an important part of the OCT treatment options.

Convalescent plasma therapy in COVID-19 patients

There is now a large body of literature about CP in COVID-19 treatment. While initial assessments of its effectiveness may have produced inconclusive findings, a thorough examination based on biological plausibility and guided by the principles of antibody therapy demonstrates that during the early stages of the pandemic, when COVID-19 had a high mortality rate, CP significantly decreased mortality when administered promptly and in high concentrations of specific antibodies[113]. In COVID-19 disease, CP may have several advantageous effects. The primary mechanism is mostly related to the ability of antibodies derived from CP to inhibit the presence of viruses in the bloodstream. Theoretically, administering CP early in the disease course would be more beneficial, much like the strategies implemented during the SARS pandemic. The majority of viral infections have a peak in viremia within the first week of infection. Typically, the host's primary immune response is established by days 10-14 of infection, indicating the elimination of the viruses[114]. Some researchers suggest that this immune response may begin slightly sooner. Additional possible processes involve antibody-dependent cellular cytotoxicity, complement activation, and phagocytosis (ADCP). Moreover, the existence of non-neutralizing antibodies that attach to the pathogens could potentially be advantageous[115].

The utility of CP in immunocompromised individuals is particularly intriguing[116]. The utilization of CP therapy may be seen as a viable alternative for immunocompromised patients, particularly in regions with restricted availability of monoclonal antibody treatments. Aside from the study by Lang-Meli *et al*[117], there is insufficient data to support this medication's safety and efficacy in patients with reduced antibody levels. In their study, the authors presented the details of 16 COVID-19 patients who had primary antibody deficiency and were treated with CP. They found that plasma administration was linked to a decrease in viral load and improvement of clinical symptoms, even when implemented seven days after the infection. Aside from a transient fever reaction in one patient, there were no other significant side effects[117]. However, since a large portion of the population has gained immunity to SARS-CoV-2 due to vaccinations and spontaneous infection, nowadays CP is no longer as helpful for immunocompetent individuals. Nevertheless, CP continues to have a crucial role in managing COVID-19 in individuals with weakened immune systems, who frequently exhibit suboptimal responses to both vaccinations and infection.

mAbs in treatment of covid-19 patients

Lessons learned from past epidemics (SARS, Ebola) paved the way for using mAb-based therapeutics in COVID-19 pandemics. Various mAbs, including bevacizumab, sarilumab, adalimumab, camrelizumab, eculizumab, mepolizumab, PD-1 monoclonal antibody, and tocilizumab, are now being studied as potential treatments for COVID-19[118]. These therapies are being examined in ongoing research. The literature data showed that focusing on the S1 subunit of the spike glycoprotein makes it possible to develop mAbs that are highly targeting, efficient, and can be easily manufactured for the treatment of COVID-19. This approach aims to specifically target the structures of SARS-CoV-2, the virus responsible for COVID-19.

Researchers are currently assessing the potential therapeutic benefit of targeting granulocyte macrophage-colony stimulating factor with mAb to mitigate lung injury or ARDS, believed to be primarily caused by lung hyper-inflammation[119]. Effects of this therapy are shown in Figure 1.

Another interesting therapeutic option, which has the potential to be beneficial, is bevacizumab, a recombinant-humanized monoclonal antibody that specifically targets vascular endothelial growth factor (VEGF). The drug can potentially decrease the levels of VEGF induced by severe inflammation, thus inhibiting the development of edema in patients suffering from COVID-19[120]. Several other mAbs, such as adalimumab, camrelizumab, eculizumab, mepolizumab, PD-1 mAb, and tocilizumab, are now being tested in clinical trials in combination with protease inhibitors[121].

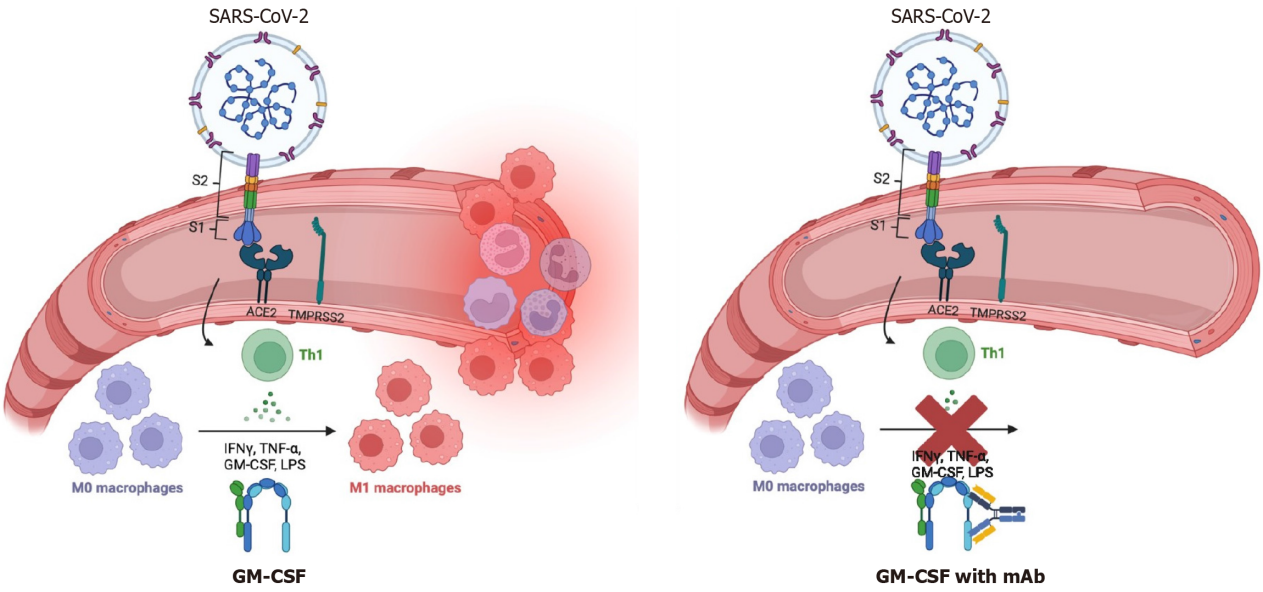

Figure 1 Blocking of granulocyte macrophage-colony stimulating factor with monoclonal antibody to cease inflammatory responses associated with proinflammatory cells, *i.e.*, M1 macrophages. SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; GM-CSF: Granulocyte macrophage-colony stimulating factor; mAb: Monoclonal antibody.

CELL-BASED THERAPIES

Some cell-based therapies discussed for COVID-19 are NK cells, T cells, CAR-T cell therapy, and stem cell therapy. It was shown that NK cells can act precisecly during immune dysfunction and cytokine storm due to their antiviral and regulatory functions[122].

A clinical trial with NK cells for COVID-19 treatment demonstrated safety and efficacy along with standard therapy. Improvements in respiratory distress and immunological parameters and decreased mortality were noted[123].

Additionally, imiquimode can enhance NK cells, further contributing to COVID-19 improvement[124].

T cell-based therapies, *i.e.*, CD4+ CD25+ FoxP3+ regulatory T-cell, anti-CD19 CAR T-cell axicabtagene ciloleucel (Brand name: Yescarta) and tisagenlecleucel (Brand name: Kymriah) were also explored for treatment in COVID-19 patients[125, 126].

To achieve the desired outcomes during the COVID-19 pandemic, Bishop stressed the importance of optimizing CAR T-cell therapy. It is possible to generate CAR T cells specific for the viral surface antigens, which can be used as therapeutic vaccines or for the targeted destruction of virally infected cells to stop the infection from spreading further within the body[127].

Hu *et al*[128] noted that CAR T-cell therapy was complicated during the COVID-19 pandemic and emphasized the importance of considering several medical and technical issues before, during, and following CAR-T therapy.

COVID-19 has shown promise for the use of stem cell therapy. Several clinical trials utilizing stem cells alone or with other treatment modalities are being researched[129,130].

The seven enrolled patients showed improvement in lung function and symptoms within two days of MSC transplantation. Clinical outcomes, inflammatory factors, immune function changes, and adverse effects were measured for 14 d after mesenchymal stem cell injection. The laboratory evaluation results showed that after 3–6 d, there was a significant decrease in TNF-α levels but an increase in IL-10 levels in the MSC treatment group compared to the placebo control group. Additionally, there was an absence of highly activated cytokine-secreting immune cells, such as CXCR3+ CD4 + T cells, CXCR3+ CD8 + T cells, and CXCR3+ NK cells[131].

We can also mention TLR therapies used to modulate innate immunity. TLR5 aids in activating innate immunity and stimulates TLR5 through bacterial flagellin, which can help in early modulation of immune response against COVID-19 and thus have therapeutic or prophylactic applications. Imiquimod aids in TLR7 activation, stimulation of specific and nonspecific immune response, and cytokine production, thus potentially being useful in COVID-19 therapy[88,124,132].

Immunomodulatory techniques may reduce lung inflammation and lower the risk of pneumonia or ARDS in an infected individual by modulating the immune response. However, as Verma *et al*[133] discussed, we must keep in mind that immunomodulatory approaches have the potential to be a double-edged sword (2023).

Therefore, it is vital to conduct extensive research on identifying new and specific prospective biological targets that may help minimize inflammation and cytokine storm. Antiviral and anti-inflammatory therapies or drugs should be administered early during COVID-19 infection due to pulmonary inflammation that can become uncontrollable and may result in immunosuppression, pneumonia, and ARDS that causes severe lung injury[134].

CONCLUSION

A common theme in regulating the detrimental effects of respiratory viruses like SARS-CoV-2 is finding ways to lessen the exacerbated inflammatory effects of the virus, which otherwise lead to respiratory failure and severe sepsis and/or shock. An astounding number of randomized trials investigating agents for COVID-19 treatment are underway, focusing on many treatment modalities. Many of the agents, as mentioned above, target the innate immune system to block proinflammatory cytokine production. These therapies are urgently needed and should be further investigated to combat a pandemic caused by a novel virus. Glucocorticoids, however, have many effects on the host immune system, including non-specific immunosuppression. Moreover, because of the glucocorticoids side effects, steroid-sparing therapy is required for COVID-19. The best agents selectively reduce an unwanted host inflammatory response without changing T-cell and monocyte-mediated antiviral activity. As a result, new therapies, like CD24Fc, have a higher safety profile than steroids and may protect better against severe COVID-19 disease by reducing the host inflammatory response without causing widespread immunosuppression.

FOOTNOTES

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Country/Territory of origin: Bulgaria

ORCID number: Hristo Valkov 0000-0003-3001-3278; Metodija Sekulovski 0000-0001-8374-7756; Russka Shumnalieva 0000-0003-2321-6536.

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MINIREVIEWS

Long chikungunya? An overview to immunopathology of persistent arthralgia

Jayme Euclydes Picasky Silveira-Freitas, Maria Luiza Campagnolo, Mariana dos Santos Cortez, Fabrício Freire de Melo, Ana Carla Zarpelon-Schutz, Kádima Nayara Teixeira

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Jayme Euclydes Picasky Silveira-Freitas, Maria Luiza Campagnolo, Mariana dos Santos Cortez, Ana Carla Zarpelon-Schutz, Kádima Nayara Teixeira, Campus Toledo, Universidade Federal do Paraná, Toledo, Paraná 85919-899, Brazil

Fabrício Freire de Melo, Instituto Multidisciplinar em Saúde, Universidade Federal da Bahia, Campus Anísio Teixeira, Vitória da Conquista, Bahia 45029-094, Brazil

Ana Carla Zarpelon-Schutz, Programa de Pós-graduação em Biotecnologia, Palotina, Universidade Federal do Paraná-Setor Palotina, Paraná 85950-000, Brazil

Kádima Nayara Teixeira, Programa Multicêntrico de Pós-graduação em Bioquímica e Biologia Molecular, Palotina, Universidade Federal do Paraná-Setor Palotina, Paraná 85950-000, Brazil

Corresponding author: Kádima Nayara Teixeira, PhD, Professor, Campus Toledo, Universidade Federal do Paraná-, Max Planck 3796, Toledo, Paraná 85919-899, Brazil.
kadimateixeira@ufpr.br

Abstract

Chikungunya fever (CF) is caused by an arbovirus whose manifestations are extremely diverse, and it has evolved with significant severity in recent years. The clinical signs triggered by the Chikungunya virus are similar to those of other arboviruses. Generally, fever starts abruptly and reaches high levels, followed by severe polyarthralgia and myalgia, as well as an erythematous or petechial maculopapular rash, varying in severity and extent. Around 40% to 60% of affected individuals report persistent arthralgia, which can last from months to years. The symptoms of CF mainly represent the tissue tropism of the virus rather than the immunopathogenesis triggered by the host's immune system. The main mechanisms associated with arthralgia have been linked to an increase in T helper type 17 cells and a consequent increase in receptor activator of nuclear factor kappa-B ligand and bone resorption. This review suggests that persistent arthralgia results from the presence of viral antigens post-infection and the constant activation of signaling lymphocytic activation molecule family member 7 in synovial macrophages, leading to local infiltration of CD4+ T cells, which sustains the inflammatory process in the joints through the secretion of pro-inflammatory cytokines. The term "long chikungunya" was used in this review to refer to persistent arthralgia since, due to its manifestation over long periods after the end of the viral infection, this clinical condition seems to be characterized

more as a sequel than as a symptom, given that there is no active infection involved.

Key Words: Key ords: Chikungunya; Immunopathology; Inflammation process; Persistent arthralgia; Signaling lymphocytic activation molecule family member 7

Core ip: This review of Chikungunya fever focuses on one of the most prevalent and important symptoms of the disease-arthralgia. The authors propose an approach to explain the persistence of arthralgia for a long time after the resolution of the infection, based on the sustained inflammatory response, mainly by macrophages and T helper type 17 cells. Additionally, it is suggested that, given the context, persistent arthralgia is a sequel of CF and could therefore be termed "long Chikungunya".

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INTRODUCTION

Chikungunya fever (CF) is a disease caused by the Chikungunya virus (CHIKV), an arbovirus of the Togaviridae family, genus Alphavirus, which has positive single-stranded RNA genetic material[1,2]. CF is transmitted primarily by mosquitoes of the *Aedes* genus, including *Aedes aegypti*, *Aedes furcifer*, *Aedes africanus*, and *Aedes albopictus*[2,3]. Transmission can occur through both urban and sylvatic cycles[4,5]. In the urban cycle, transmission occurs between humans and mosquito vectors, while the sylvatic cycle involves non-human primates[5,6]. In addition to transmission through mosquito bites, transmission through blood transfusions is also possible in the urban cycle[7]. The possibility of vertical transmission from an infected mother to fetus and sexual transmission has also been proposed. Sexual transmission has not yet been confirmed, and viruses have only been found in semen[4,6,8].

Since its first recorded appearance in 1952 in Tanzania, Africa, CHIKV has garnered attention for its recurrence, with outbreaks occurring every 7-20 years. In 1958, CHIKV began to be reported in central and southern regions of Africa (Uganda and the sub-Saharan region), followed by outbreaks in Asia between 1958 and 1973, and Kenya in 2004. From Kenya, it spread to the Indian Ocean, India, and Southeast Asia, resulting in more than 6 million cases. Recent outbreaks have been notable for the high number of infected individuals. In addition to the outbreak in India, Comoros recorded 215000 cases of the disease in 2005, with a further 255000 cases reported on Reunion Island, east of Madagascar, between 2005 and 2006[2,9]. Since 2007, health agencies have increased their focus on CF and its causative agent due to its spread to regions of the world previously unaffected[2,10].

In addition to the significant number of cases and its ability to spread, the occurrence of fatal cases contrasts with CF's typical status as a self-limiting and mild disease. Reports of new modes of CF transmission, such as vertical transmission, have also contributed to increased awareness of the disease, which has regained attention recently due to the exponential advancement of global warming. This partly explains the presence of CHIKV in regions previously less affected, such as Europe and the Americas[8,11,12].

In Brazil, especially following the 2014 outbreak in the Northeast region, an association was observed between CF and other arboviruses-Zika and Dengue virus. This association indicates not exacerbation in CF cases, but rather complications in dengue cases leading to hospitalizations[13].

Much of the impact caused by CHIKV and CF stems from the virus's ability to easily adapt to new locations, owing to its capacity to attract new species of anthropophilic vectors[12].

The repercussions of CF extend beyond the realm of health, affecting the economy, social welfare, and other areas. A qualitative study conducted in Curaçao revealed that the social impacts of CF varied depending on the manifestation, duration, and severity of the disease. Patients reported social isolation, inability to engage in physical and daily activities due to physical limitations. Regarding emotional impact, there were reports of stress, anxiety, shame, frustration, despair, feelings of social exclusion, and even personality dissociation. No significant financial impacts were observed, as Curaçao has a public health system that mitigated the damage[14].

CF has an incubation period of 3 to 7 d[2,3,15]. There are three clinical stages defined in CF: The acute stage from the 1st to the 21st d of infection; post-acute stage from the 21st d to the 3rd month, and chronic stage from the 3rd month onwards[16]. Occasionally, infections can be asymptomatic, but these represent a minimal proportion of infected individuals[2,3].

Serological data can indicate leukopenia, thrombocytopenia, and elevated levels of liver transaminases[17]. Some possible findings in individuals experiencing arthralgia include joint effusion, bone erosion, spinal cord edema/erosion, synovial thickening, tendonitis, and tenosynovitis, which can be detected by nuclear magnetic resonance imaging[18].

Clinical manifestations can be acute or chronic. During the acute phase, symptoms may include arthralgia, with or without fever exceeding 38.9 °C, low back pain, headache, fatigue, oligoarthralgia, or polyarthralgia (typically bilateral, predominantly affecting large and peripheral joints such as knees, ankles, wrists, shoulders, and phalanges), ocular hyperemia, oral ulcers[2,3,19], macular or maculopapular skin lesions that are swollen or pruritic, typically affecting the palms of the hands, soles of the feet, torso, and face.

Gastrointestinal symptoms such as nausea, vomiting, and diarrhea may also be present during the acute phase, along with erythema, asthenia, conjunctival effusion, persistent conjunctivitis, and cervical lymphadenopathy, though the latter is less common. The severity and presence of these symptoms are associated with viral load, considered high when it ranges from 105 to 109 copies of viral RNA per milliliter[8], as well as age and biological sex[2,19].

Other atypical manifestations associated with CF have also been reported, such as Guillain-Barré syndrome, partial or total alopecia (predominantly in women), uveitis, and retinitis. Fever, loss of appetite, apnea, skin manifestations, distal and cerebral edema, encephalitis, hemorrhage, cardiac symptoms (myocarditis)[2,20], respiratory issues (Acute respiratory distress syndrome), renal complications (rhabdomyolysis, acute interstitial nephritis, thrombotic microangiopathy, and kidney damage)[20], and gastrointestinal symptoms have been reported in vertically infected neonates.

In infants, bullous lesions have been reported on the second day following the febrile state. There have also been reports of deaths due to CF in neonates, immunocompromised individuals, and the elderly, possibly linked to neurological disorders[21], such as encephalitis, encephalopathy, cognitive disorders, mood swings, depression, confusion, and memory loss[8,20].

During the chronic phase of CF, the main symptom is persistent arthralgia, which, when it appears after the acute phase of the disease (7-10 d)[2], can last from weeks to years, depending on the affected population, age, and the presence of comorbidities, affecting both peripheral and large synovial joints[2,8]. Additionally, alopecia, depression, mood swings, sleep disturbances, blurred vision, and memory loss have also been associated with the chronic phase of CF[22].

Initially, CHIKV remains present in the blood and lymph, characterizing the acute phase of CF. As the disease progresses, other organs and cell types become infected due to the hematogenous distribution route. Cell types include natural killer (NK) cells, T and B cells, dendritic cells, macrophages, synovial fibroblasts, endothelial cells, and myocytes.

The chronic condition of CF can mimic arthritis, particularly rheumatoid arthritis, with symptoms such as joint effusion, bone erosions, medullary edema, synovial thickening, tendonitis, and tenosynovitis, which are present in around 55% to 65% of cases. Among these cases, 90% reported bilateral joint involvement, 63% reported joint edema, and 39% experienced chronic myalgia. Middle-aged individuals and women are predominantly affected[23].

Regarding CF, studies indicate that the persistence of chronic symptoms is sustained by a prolonged inflammatory response[24]. In rheumatoid arthritis, high levels of interleukin (IL) 12 are found, which is responsible for bone degradation and, consequently, arthralgia. Similarly, CHIKV induces the proliferation of osteoclasts by proliferating in synoviocytes[21,25].

Despite being a serious disease in many respects, there is still no vaccine, and pharmacological treatment is neither specific nor effective, as it currently relies on symptom management using non-steroidal anti-inflammatory drugs in conjunction with corticosteroids and antipyretics[3,7,8,26-28].

Patients with polyarthralgia may present with other associated manifestations, characteristic of the continuation of an inflammatory process, such as swelling, and may therefore require corticosteroid therapy[26]. Due to the clinical similarity between the arthralgia experienced by patients infected with CHIKV and rheumatoid arthritis, treatment typically involves the use of disease-modifying anti-rheumatic drugs, such as methotrexate, sulfasalazine, leflunomide, and hydroxychloroquine[3,28]. In cases where there is an inadequate response to methotrexate, satisfactory therapeutic outcomes have been reported with the administration of tumor necrosis factor (TNF) blockers, such as etanercept[28]. One challenge in developing drugs against CHIKV is the virus's intrinsic ability to mutate, potentially leading to resistance to antivirals[29]. In fact, the exact immunological mechanisms involved in CF symptoms need to be better understood so that targeted management and treatment can be devised in search of more effective results.

CHIKV INFECTION CHRONICITY

CHIKV can be classified as an arthritogenic virus, similar to other clinically relevant alphaviruses, as it typically induces debilitating musculoskeletal diseases characterized by myalgia, arthralgia, and arthritis[30]. It is estimated that 30% to 60% of infected individuals develop long-term sequelae, with symptoms persisting for several years[3,31-33]. Over a third of patients report persistent or recurrent polyarthralgia, with approximately half of them experiencing chronic rheumatic manifestations[34,35].

In preclinical infection trials, it was observed that CF symptoms primarily reflect the tissue tropism of the virus rather than the immunopathogenesis triggered by the host's immune system. These trials demonstrated that the severity of the infection is directly linked to the inefficiency of type I-interferon (I-IFN) signaling[36]. Another study revealed the presence of type I-IFN in the synovial tissue of patients with chronic CHIKV infection[37].

Fibroblasts are the primary targets of CHIKV, and as these cells are found in tissues such as joint capsules, fascia, and muscle insertions, they account for the pronounced intensity of myalgia reported by patients, as these structures contain numerous nociceptive nerve endings[36]. Additionally, there is viral tropism for blood monocytes and joint macrophages, directly contributing to the initiation of an inflammatory process during acute infection[5,37].

In studies conducted on non-human primates, the CHIKV genome was identified in splenic macrophages approximately 3 months post-infection, suggesting the significance of macrophages in viral persistence. Furthermore, the prolonged presence of viral antigens in lymph nodes, liver, and muscles supports the notion that macrophages act as

crucial viral reservoirs[38]. Additionally, viral tropism towards muscle satellite cells has been observed in ex vivo and in vitro studies[39].

Viral persistence has been associated with the inefficiency of the host's immune system and the effectiveness of viral evasion mechanisms[37]. In a human study, the severity of the infection was found to be correlated with elevated serum levels of pro-inflammatory cytokines, such as IL-6 and IL-1β, along with a decrease in regulated on activation, normal T cell expressed and secreted (RANTES) levels, also referred to as C-C motif ligand (CCL) 5. During the acute phase, an inflammatory pattern predominates, driven by a Th1 immune response, with circulating cytokine levels even more pronounced in individuals with high viral loads. However, as the infection progresses to symptomatic stages, there is a shift towards Th2 response markers, such as IL-7, IL-10, and IL-15[40,41].

The identification of IL-7 and IL-15 in the tissue and synovial fluids of patients with rheumatoid arthritis suggests that these cytokines may be implicated in the development of arthralgia associated with CHIKV infection[40,42,43]. IL-15 produced by synoviocyte fibroblasts induces the expression of IL-17, which has been linked to the pathogenesis of rheumatoid arthritis and the chronic phase of CHIKV infection[41,43]. Osteoblast infection is also observed in patients with chronic arthritis, leading to detrimental effects on bone mineralization due to the inhibition of osteoprotegerin by IL-6 present in infected joints[3,5,31].

Persistent arthralgia typically emerges after the resolution of the acute CF phase, and despite its chronic nature, little is known about the mechanisms and factors contributing to its progression[3,5,18], although various studies suggest different risk factors. Patients with chronic arthralgia exhibit fibroblast hyperplasia, angiogenesis, tissue damage due to elevated levels of metalloproteinase-2, cell death, and infection of perivascular macrophages in synovial tissue[37].

Persistent arthralgia resulting from CHIKV infection also demonstrates elevated serum concentrations of IL-1Ra, IL-1β, IL-6, IL-7, IL-8, IL-12, IL-15, and IFN-α, including IL-17, a cytokine prominent in rheumatoid arthritis; this indicates that the persistent arthralgia of CF physiologically resembles rheumatoid arthritis. However, unlike rheumatoid arthritis, which exhibits serum levels of anti-cyclic citrullinated peptide) and anti-rheumatoid factor antibodies, along with an increase in the CCL5/RANTES chemokine ratio, the persistent arthralgia of CF is characterized by the presence of anti-CHIKV IgM or IgG, a decrease in the CCL5/RANTES ratio, and an increase in granulocyte macrophage-colony stimulating factor (GM-CSF) and TNF-α[23]. An *in vivo* study comparing serum levels of TNF-α, IL-13, IL-2, and IL-4 during acute infection in patients who developed chronic arthralgia and those without persistent manifestations over a 20-month period post-infection observed that an intense cytokine response during the acute phase led to a reduced incidence of chronic arthralgia, whereas low cytokine levels were associated with pronounced chronic joint pain[27].

ARTHRALGIA TRIGGERED BY CHIKV

The immunopathogenic mechanisms of CF are akin to those responsible for the immune response against CHIKV. Acute CHIKV infection is characterized by elevated serum levels of pro-inflammatory chemokines, such as CCL2, CCL4, C-X-C motif chemokine ligand (CXCL) 9, and CXCL10, as well as cytokines IFN type 1, IFN-γ, IL-6, IL-8, IL-17, growth factors, and GM-CSF[31]. This inflammatory microenvironment triggers a robust migration of phagocytic cells such as macrophages and activation of CD8+ T cells and NK cells to eradicate the viral agent[44,45]. Although macrophages are pivotal for the protective response, their presence in the joints contributes to the inflammatory process[46].

The number of effector lymphocytes increases during the pathogenic process of CF, and this augmentation correlates with the onset of arthralgia[47]. There is a differentiation of T cells into the Th17 subtype, observed in animal models of rheumatoid arthritis and associated with pain manifestation in clinical settings[23]. Thus, Th17 polarization leads to elevated circulating concentrations of IL-17, linked to bone matrix destruction and stimulation of other cytokines, pro-inflammatory chemokines, and matrix metalloproteinases (MMPs), promoting cartilage degradation. Indeed, MMP2 was found in high levels in the synovial fluid of patients with chronic arthralgia[48,49].

Therefore, IL-17 is crucial for arthralgia development, playing a role in bone resorption and weakening through receptor activator of nuclear factor kappa-B ligand (RANKL) expression, which binds to RANK and regulates osteoclast differentiation, thereby increasing bone resorption and exacerbating joint pain[50]. It is hypothesized that the increase in CD4+ T cells in the joint inflammatory microenvironment may worsen the condition due to intense TNF-α release, as this cytokine contributes to the pathogenesis of psoriatic arthritis and rheumatoid arthritis[51-53].

Studies suggest that NK cells are also involved in acute arthralgia caused by CHIKV in murine models[54-56], as the increased presence of these cells in the synovial region correlates with the arthralgic mechanism in rheumatoid arthritis through the action of TNF-α and IFN-γ, although this mechanism is not fully understood[55,57]. Granzyme A, released by CD8+ T cells and NK cells, also plays a significant role in CHIKV-induced arthralgia[58]. Granzyme A promotes the degradation of type IV collagen and lymphocyte migration to the synovial joint[59-61]. This association is underscored by higher serum levels of granzyme A in CHIKV-infected individuals compared to uninfected individuals, along with its proteolytic and pro-inflammatory activity[55,60,62-64] (Figure 1).

IMMUNOPATHOLOGY OF PERSISTENT ARTHRALGIA

Some proposals attempt to explain the chronic pathogenesis of CHIKV in the joints. The establishment of the virus, through tissue tropism, in fibroblasts, satellite cells, and myoblasts[36,39,65] makes these cells important reservoirs for the persistence of viral antigens, such as viral RNA, in the affected organism, even after the acute infection has resolved[37,38,66]. The presence of these antigens in the joints may be one of the causes of persistent arthralgia, although the

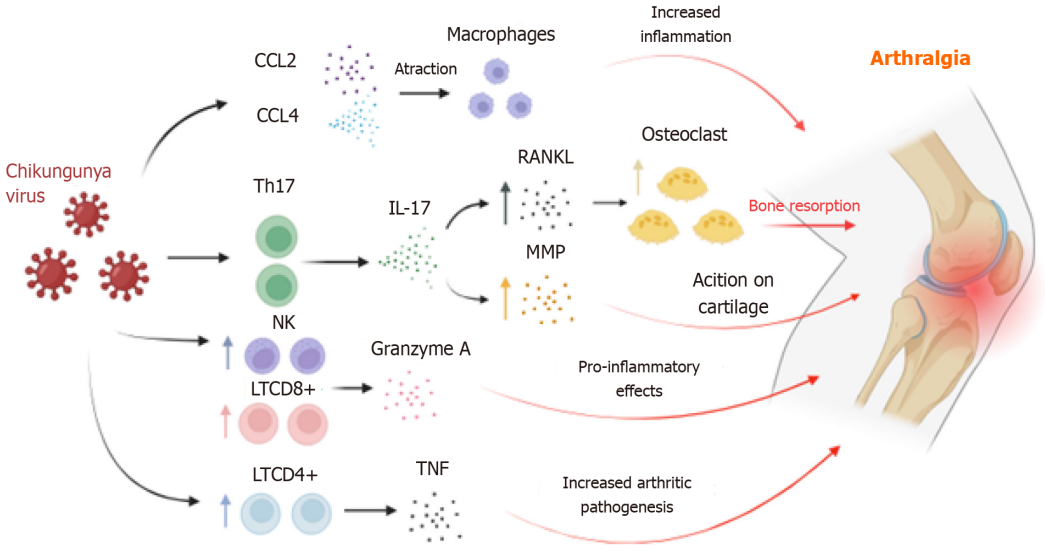

Figure 1 Supposed mechanisms involved in the pathogenesis of arthritis caused by Chikungunya virus. The elevation in levels of pro-inflammatory chemokines induces T helper type 17 response polarization, leading to interleukin-17 expression and subsequent increase in receptor activator of nuclear factor kappa-B ligand. Granzyme A released by natural killer cells and CD8+ T cells targets type IV collagen, contributing to progressive joint damage. Th17: T helper type 17; CCL4: C-C motif ligand 4; NK: Natural killer; LTCD8: CD8 T lymphocytes; LTCD4: CD4 T lymphocytes; RANKL: Receptor activator of nuclear factor kappa-B ligand; MMP: Matrix metalloproteinases; IL: Interleukin; TNF: Tumor necrosis factor.

mechanisms are not fully understood. In addition to antigenic persistence, the presence of T cells in the joints, in a chronic state, can be correlated with an increase in local IL-17 levels and other pro-inflammatory cytokines, which exacerbate joint pain[67]. The actions of IL-17, along with RANKL, are related to the development of arthralgia, as previously mentioned [23,41,68-70].

While macrophages are essential phagocytes for controlling numerous microbial infections, they can contribute to an increase in acute inflammation, directing it towards a chronic state and causing tissue damage[71,72]. Dysfunctional macrophages are commonly found in autoimmune diseases such as rheumatoid arthritis[73,74]. Macrophage overactivation occurs through receptors and signaling molecules in the infectious microenvironment, and some studies have shown that the signaling lymphocytic activation molecule family member 7 (SLAMF7) receptor or CD139 plays a crucial role in transforming these phagocytic cells into an explosive, highly inflammatory, and potentially pathogenic state[75,76].

In an unstimulated state, the SLAMF7 receptor may be expressed in plasma cells, NK cells, B and T cells, albeit at low levels in macrophages[77,78]. In vitro treatment of macrophages with IFN-γ has been shown to increase SLAMF7 expression, as well as IFN-β and TNF-α[76]. IFN-γ has been identified as a key regulator of SLAMF7; activation of the SLAMF7 receptor by r-SLAMF7 protein led to up-regulation of TNF-α, IL-1β, IL-6, CCL3, CCL4, CXCL1, CXCL2, and CXCL8, suggesting an intrinsic up-regulation feedback loop. Additionally, an increase in IL-6 and TNF-α levels was observed after stimulation[76].

The initial SLAMF7 activation sequence involves IFN-γ expression. IFN-γ potentiates and increases the number of SLAMF7 receptors on the surface of macrophages, and after the initial stimulus, receptor engagement triggers a highly potent activation of the inflammatory state in these cells[76]. Furthermore, after SLAMF7 induction by IFN-γ, TNF-α from the microenvironment can recruit molecules from autocrine amplification pathways. This suggests that TNF-α participates in an additional step in the maintenance of the inflammatory process[76].

During the acute viral infection phase, the host immune system responds to the infection by releasing IFN-γ by NK cells, antigen-presenting cells, and B cells; in addition to acting in an autocrine and paracrine manner in Th1 cell differentiation, intervening in viral replication[79-81]. IFN-γ release, along with macrophage/monocyte chemotaxis by the chemokines CCL2 and CCL4, may lead to increased SLAMF7 receptor expression in phagocytic cells that have migrated to the inflammatory site[46,76,82].

Thus, in the long term, the activation of SLAMF7 receptors in synovial macrophages associated with the persistence of viral antigens promotes the constant presence of activated CD4+ T cells in the joints; together with macrophages, T cells lead to an increase and continuous release of TNF-α, which in turn acts in an autocrine manner to amplify inflammatory signaling pathways[47,76,83-86]. This condition contributes to a state of constant inflammatory activation, perpetuating the release of cytokines that allow the configuration of an inflammatory joint microenvironment, resulting in greater tissue damage and clinical worsening (Figure 2).

CONCLUSION

The arthralgia is one of the most prevalent and relevant symptoms of CF in both the acute and chronic phases of the disease. The fact that arthralgia is reported by individual months and even years after the infection has resolved charac-

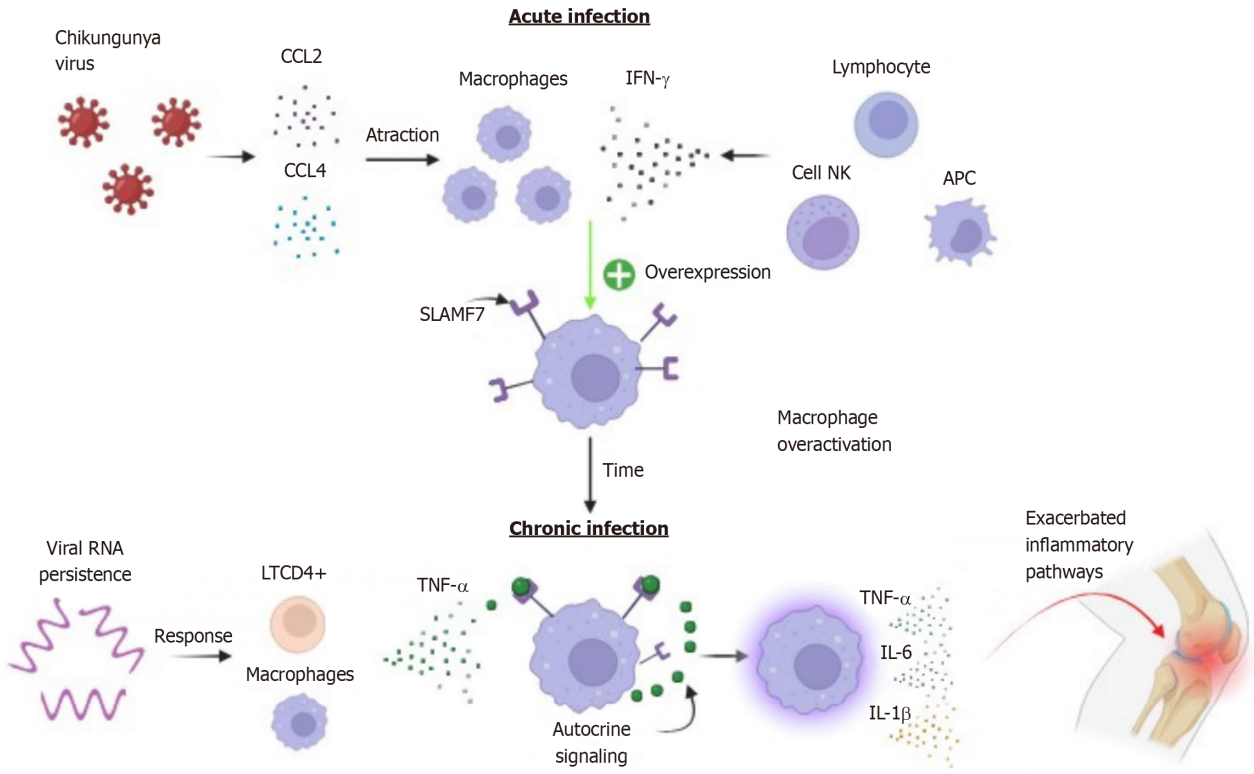

Figure 2 Hypothesis for persistent arthralgia caused by Chikungunya virus infection. During the initial viremia phase, interferon gamma stimulates increased expression of the signaling lymphocytic activation molecule family member 7 receptor in macrophages, which persists into the chronic phase due to continuous stimulation by Tumor necrosis factor-alpha. Activation of the signaling lymphocytic activation molecule family member 7 receptor and persistence of viral antigens (RNA) enable macrophages to sustain a state of chronic inflammatory over-activation, perpetuating the release of cytokines that facilitate the formation of an inflammatory microenvironment, tissue damage, and clinical deterioration. SLAMF7: Signaling lymphocytic activation molecule family member 7; LTCD4: CD4 T lymphocytes; CCL: C-C motif ligand; NK: Natural killer; TNF-α: Tumor necrosis factor-alpha; IL: Interleukin; APC: Antigen presenting cells; IFN-γ: Interferon gamma.

terizes this manifestation as persistent. Although arthralgia was triggered by the viral infection, persistent arthralgia is not supported by the presence of the infection. In this context, persistent arthralgia, in the absence of infection, classifies this clinical condition as a sequel rather than a symptom. Therefore, in comparison to similar cases involving viral agents, this post-infection condition could be determined, for the first time, as "long chikungunya".

FOOTNOTES

Author contributions: Silveira-Freitas JEP, Campagnolo ML, dos Santos Cortez M collected the data and wrote the manuscript; Melo FF performed the critical analysis of the manuscript; Zarpelon-Schutz AC and Teixeira KN performed the critical analysis, corrected the manuscript and coordinated the study. All authors approved the final version of the manuscript.

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Country/Territory of origin: Brazil

ORCID number: Jayme Euclydes Picasky Silveira-Freitas 0000-0002-3249-6536; Maria Luiza Campagnolo 0009-0003-4600-3143; Mariana dos Santos Cortez 0000-0001-6518-0792; Fabrício Freire de Melo 0000-0002-5680-2753; Ana Carla Zarpelon-Schutz 0000-0002-8320-3633; Kádima Nayara Teixeira 0000-0002-2928-9181.

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MINIREVIEWS

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Mohamad Saifudin Hakim, Postgraduate School of Molecular Medicine, Erasmus MC-University Medical Center, Rotterdam 3015GD, Netherlands

Mohamad Saifudin Hakim, Viral Infection Working Group, International Society of Antimicrobial Chemotherapy, London EC4R 9AN, United Kingdom

Faris Muhammad Gazali, Master Program in Biotechnology, Postgraduate School, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia

Suci Ardini Widyaningsih, Master of Medical Sciences in Clinical Investigation, Harvard Medical School, Boston, MA 02115, United States

Mohammad Khalid Parvez, Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, 11451, Saudi Arabia

Corresponding author: Mohammad Khalid Parvez, PhD, Professor, Department of Pharmacognosy, College of Pharmacy, Building 23, Room 35-2B, King Saud University, PO Box 2457, Riyadh 11451, Saudi Arabia. mohkhalid@ksu.edu.sa

Abstract

Rotaviruses are non-enveloped double-stranded RNA virus that causes acute diarrheal diseases in children (< 5 years). More than 90% of the global rotavirus infection in humans was caused by Rotavirus group A. Rotavirus infection has caused more than 200000 deaths annually and predominantly occurs in the low-income countries. Rotavirus evolution is indicated by the strain dynamics or the emergence of the unprecedented strain. The major factors that drive the rotavirus evolution include the genetic shift that is caused by the reassortment mechanism, either in the intra- or the inter-genogroup. However, other factors are also known to have an impact on rotavirus evolution. This review discusses the structure and types, epidemiology, and evolution of rotaviruses. This article also reviews other supplemental factors of rotavirus evolution, such as genetic reassortment, mutation rate, glycan specificity, vaccine introduction, the host immune responses, and antiviral drugs.

Key Words: Rotavirus; Epidemiology; Evolution; Reassortment; Vaccine

Core Tip: Recurrent outbreaks of human pathogenic viruses resulting in epidemics or pandemics are due to their ability to rapidly evolve and adapt as compared to the other microbial pathogens. Rotaviruses are segmented, dsRNA viruses that mainly cause acute gastroenteritis in children (< 5 years). Rotavirus evolution, especially a dynamic replacement of circulating rotavirus A from one strain into another, has been observed globally. In this review, we discuss the driving factors of rotavirus evolution, including vaccines and host-immune responses, towards improving our understanding of the evolutionary dynamics of its emerging strains as a foundation for developing effective preventive and therapeutic measures.

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INTRODUCTION

Recurrent outbreaks of human pathogenic viruses resulting in epidemics or pandemics are due to their ability to rapidly evolve and adapt as compared to the other microbial pathogens[1]. Generally, novel viruses or their new strains emerge when humans are exposed for the first time, to an evolved virus of zoonotic origin. Compared to DNA viruses, RNA viruses have a much more recent history of 'genetic-evolution' due to very high replication-fidelity rate (approximately 10^{-4} error/site/cycle) of their RNA-dependent RNA polymerases (RdRp) and therefore, 'human-adaptation[1,2]. In the evolutionary and adaptive process of RNA viruses, genetic mutations, re-arrangement or assortment, and virus-host genetic recombination are the major events towards establishing new and stable strains. Therefore, it is very much expected that such newly human-adapted strains would persist in specific populations (endemics) or spill across populations (epidemics) or eventually spread globally (pandemics[2]).

Rotaviruses are segmented, double-stranded RNA (dsRNA) viruses that mainly cause acute gastroenteritis or diarrhoeal disease in pediatric population (< 5 years). Of the nine species of rotavirus (group A, B, C, D, F, G, H, I and J), rotavirus A (RVA) primarily infects humans[3]. Six strains of RVA, *G1P[8]*, *G2P[4]*, *G3P[8]*, *G4P[8]*, *G9P[8]*, and *G12P[8]* have been shown to dominate about 90% of global rotavirus transmission in humans[4,5]. In 2019, there were 9.1% of under-five global mortality due to diarrhea as clinical presentation of rotavirus infection[6]. The mortality caused by rotavirus infection is predominantly occurred in the developing countries with middle to low income[7].

Rotavirus evolution, especially a dynamic replacement of circulating RVA from one strain into another, has been observed in Asia, Africa, Australia, America, and Europe[8,9]. In addition, the emergence of the unprecedented strains as the results of intra- or intergenogroup combinations which subsequently become the predominant strains, were also observed[10]. This genotype alteration was created by the mechanism of reassortment between strains with a similar genotype constellation[11]. However, many novel rotavirus strains have emerged by the mechanism of inter-genogroup multiple reassortment[8,12].

In addition to achieving the lower incidences of rotavirus associated-acute gastroenteritis, vaccination is also an important driving factor for the dynamic evolution of rotaviruses. The vaccine will exert selection pressure on the rotavirus genotypes and consequently, trigger the emergence of novel strains through reassortment (genetic shift)[13]. The accumulation of point mutations (genetic drift) may also reduce the effectiveness of rotavirus vaccines[14]. Furthermore, the *RVA P*[II], which has a wider host range due to glycan specificity, is known to have evolved from *RVA P*[I][15]. This event also shows the zoonotic mechanism of *RVA*[16].

Other factors that also influence the evolution of rotaviruses are the host immune responses[17]. RVA has a specific strategy to evade the innate and adaptive immune responses[18,19]. This strategy is essentially required by rotavirus to continue to survive and is the key to its continuous evolution[18]. Although no anti-rotavirus drug has yet been approved by the World Health Organization (WHO), this antiviral drug could also be a driving factor in the evolution of rotaviruses. The pressure exerted by antiviral drugs may trigger accumulation of mutations or reassortment of circulating rotavirus strains[20-23]. In this review, we discuss the driving factors of rotavirus evolution, including vaccines and host-immune responses, towards improving our understanding of the evolutionary dynamics of its emerging strains as a foundation for developing effective preventive and therapeutic measures.

GENOMIC STRUCTURE OF ROTAVIRUSES

Rotaviruses are a non-enveloped RNA virus that has 11 segments of dsRNA encapsulated by three-layered capsid proteins (Figure 1). Rotaviruses belong to the *Reoviridae* family. The segmented dsRNA genome of rotaviruses encodes six structural viral proteins (VP, VP1-VP6) and six non-structural proteins (NSP, NSP1-NSP6). The VPs are present in the mature viral particle and determine the specificity of rotaviruses, along with their capacity to induce the host immune responses[4]. The NSPs regulate the specific functions for genomic replication as well as antagonistic functions toward the host innate and adaptive immune responses[24].

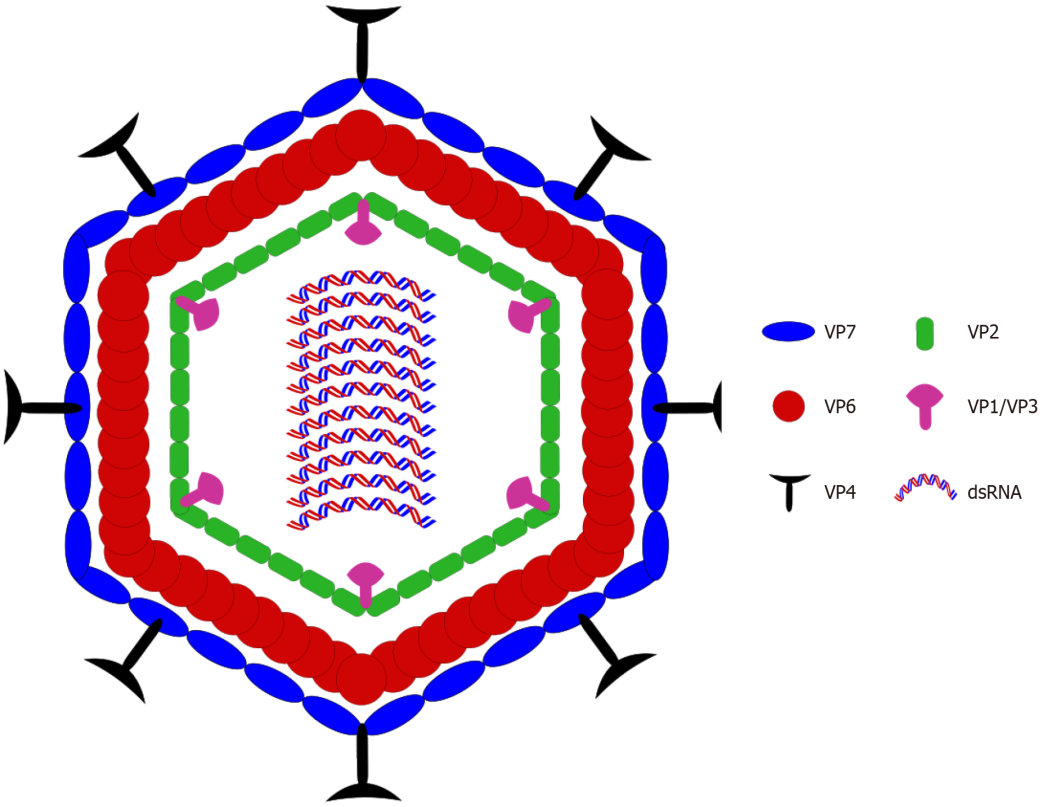

Figure 1 Schematic structure of rotaviruses. Rotaviruses are a non-enveloped RNA virus that has 11 segments of double-stranded RNA (dsRNA) covered by three layers of capsid proteins. The inner layer consists of VP1 and VP2 proteins, the middle layer consists of VP6 protein, while the outer layer consists of VP4 and VP7 proteins.

Rotaviruses are classified based on the nucleotide sequence as well as antigenic epitopes in the VP6 protein. To date, there are 10 species of rotaviruses, namely species A-J. The most common species causing infection in children is RVA. RVA can be further classified according to the RNA sequences in the RNA segments 7 and 4, which are responsible for encoding the VP7 and VP4 proteins, respectively. VP7 is a glycoprotein and is employed to classify the G-type of rotavirus, while VP4 is a protein cleaved by a protease (protease-cleaved protein) and is employed to classify the P-type of rotavirus. To date, 41 G genotypes and 57 P genotypes of rotaviruses have been successfully identified[4,25].

However, there are about six G-types and three P-types of RVA which are predominantly found circulating worldwide, namely *G1*, *G2*, *G3*, *G4*, *G9*, and *G12*, as well as *P[4]*, *P[6]*, and *P[8]*. Of the many combinations between different G and P genotypes that have been identified, there are 6 strains of RVA that play a dominant role in more than 90% of the global rotavirus spread, namely *G1P[8]*, *G2P[4]*, *G3P[8]*, *G4P[8]*, *G9P[8]*, and *G12P[8]*[4].

Classical binomial genotyping of rotaviruses into G (VP7) and P (G4) genotypes which is commonly used for rotavirus surveillance limits our knowledge on the rotavirus genome characteristics. Thus, a whole-genome classification system based on their 11 RNA segments has been introduced[26]. The segment of the RVA genome that encodes VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/NSP6 will be expressed using the abbreviations *Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx*, where *x* is the genotype number, an Arabic number starting from 1. The RVA grouping based on the 11 genome segments therefore, forms a constellation of viral genotypes that distinguishes between its various strains[9].

EPIDEMIOLOGY OF ROTAVIRUSES

In general, rotaviruses infect children under five years old and are transmitted by the fecal-oral route[4]. However, rotavirus infection predominantly occurs in children under two years of age, with the highest incidence in infants aged 7-12 months. Maternal antibodies obtained from the mother can only protect the baby from viral infection for up to 6 months and will decrease significantly thereafter. This is the main reason why the incidence of rotavirus infection often occurs in children older than six months old. The incidence of rotavirus infection decreases in children of over two years age because of increased exposure to infections and stronger immunity[27].

A total of 115 million cases of rotavirus infection in children aged less than five years were reported in 2003, with 2.3 million of whom required hospitalization. In 2013, the global mortality of children under-five years of age due to rotavirus infection reached 215000 deaths. Of this large mortality rate, more than 95% occurred in developing countries, such as countries located on the African and Asian continents[27]. During the period of 2019, 9.1% of under-five mortality in the world was caused by diarrhea as manifestation of rotavirus infection[6]. In one of Asia countries, such as Indonesia,

mortality case due to diarrhea in 2020 among children aged 29 days until 11 months and 1 to 4 years old were 9.8% and 4.5%, respectively[28]. The Global Rotavirus Surveillance Network under the coordination of the WHO reported that before the introduction of the rotavirus vaccine, nearly one-third of hospitalized diarrheal patients were caused by rotavirus infection[7]. After implementation of rotavirus vaccine into the national immunization program, the prevalence of rotavirus infection among children under-five who were hospitalized or admitted in emergency department declined by approximately 40%[29].

Rotavirus infection in children (< 5 years) causes acute diarrhea, dehydration, electrolyte imbalance, and metabolic acidosis. In addition, rotavirus infection also causes vomiting and fever, and can lead to death if not appropriately treated [27]. Children (< 5 years) in low-income countries have a higher percentage of deaths from rotavirus infection than children in high-income countries. This can be caused by several factors, such as limited coverage of health care centers, lack of hygiene and sanitation, the absence of rehydration therapy, as well as the influence of other diseases, such as malnutrition[4].

Rotavirus infection is generally non-seasonal, but the incidence rate can increase in certain seasons. In subtropical countries, for instance countries in Asia and Africa, rotavirus infection is erratic and varies throughout the year. However, the incidence rate of rotavirus infection increased significantly during the wet and dry seasons in some subtropical countries, *e.g.*, Benin, West Africa. Meanwhile, rotavirus infection in countries on the European continent tends to depend on the season where the incidence rate of rotavirus infection will increase in the winter[27].

Diarrheal disease due to rotavirus infection in a certain area is often caused by infection with rotavirus strains that are rarely found, for example the *G9P[6]* strain which prevalence is only 9.5% in India[4]. The selection pressure on the spread of natural rotavirus strains by the introduction of the vaccine will cause genotype shifting through the reassortment process. However, changes in the rotavirus genotype are not always associated with the introduction of the vaccine. For example, there were genotype changes that occurred in Indonesia which had not implemented the national (universal) rotavirus immunization program. There was a change in the genotype of the horse (equine-like strain) *G3P[8]/[6]* into typical human rotavirus strains *G1P[8]/[6]* and *G2P[8]/[6]*[30].

During the coronavirus disease 2019 (COVID-19) pandemic, there were reports on decreasing vaccination coverage against rotavirus[31]. This trend was due to disruption of routine childhood immunisation during the pandemic[32]. However, nonpharmaceutical interventions implemented during the pandemic have significantly impacted on decreasing the prevalence of rotavirus infection in children[33,34]. Interestingly, water-based surveillance in Japan of enteric viruses during the COVID-19 pandemic, identified rotavirus as the most frequently detected viral pathogen, indicating its continuous transmission in the community[35]. A modeling study further predicted re-surgence of rotavirus in post-pandemic period; however therein, a thorough epidemiological study is needed to confirm the findings[36].

GENETIC REASSORTMENT, MUTATION RATE, AND GLYCAN SPECIFICITY

Genetic reassortment (genetic shift)

The segmented genomes of rotaviruses can derive from two different mechanisms, either through the process of accidentally exchanging two genomes from two different strains (genetic reassortment), or through the process of diploidy or polyploidy where more than one genome have been randomly packed into the viral progeny. Rotaviruses have a unique characteristic, which is their ability to sort and exchange its genetic material during the co-infection process of two different strains, known as the reassortment. These viruses are able to coinfect a single host cell, then exchange their segmented RNA genetic materials, resulting in a single "hybrid" virion. The reassortment process in RVA is the main mechanism for the virus to evolve and increase its diversity[11]. Reassortment is best-described in influenza virus as a primary mechanism for interspecies (animal-to-human) transmission and the emergence of novel pandemic strains[37].

The reassortment mechanism of RVA has not yet to be comprehensively elucidated due to the limitations of *in vitro* experimental systems. However, available data indicate that there is a similar process between the reassortment mechanism of RVA with ϕ6 *(Pseudomonas phage phi6)* and influenza A viruses. Reassortment in RVA begins with the transcription of double-stranded RNA (dsRNA) by viral polymerases which produces positive-sense RNA. A total of 11 positive segments of RNA that have been transcribed will form a complex of supramolecular structures and be immediately assembled by virion particles that grow through the encapsidation process. The polymerase enzyme then converts positive RNA into dsRNA shortly after the encapsidation process takes place (Figure 2)[11].

The reassortment pattern of RVA is strongly influenced by the constellation of the genome[38]. In general, the RVA genome is divided into three constellations of genotypes: Genogroups 1, 2, and 3. Genogroup 1 or Wa-like has a genotype constellation *G1/3/4/9/12-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1*; genogroup 2 or DS-1-like has the genotype constellation *G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2*; and genogroup 3 or AU-1-like has a genotype constellation *G3-P[9]-I3-R3-C3-M3-A3-N3-T3-E3-H3*[12]. Reassortment that occurs in intra-genogroups will produce reassortants that have variations at the subgenotype level[39,40]. Rotavirus reassortment rarely occurs across genogroups because the viral progeny tends to have low survival. This can be influenced by the possible incompatibility of RNA or protein encoded by RNA segments in viruses that undergo reassortment across genogroups. Consequently, it will lead to the constraints in viral replication [11]. Interestingly, full-genome characterization of rotaviruses from 2022-2017 in Vellore, India showed that most strains had stable classical genotype constellation of Wa-like and DS-1-like and only a small number had reassortant constellations. A similar finding was reported from 6-year surveillance (2010-2016) of rotaviruses in northern Brazil[41].

Although the evolution of rotavirus is highly dependent on reassortment associated with the constellation of the genome, several studies have reported that there are several types of RVA identified to have evolved through intergenogroup reassortments. RVA strain DS-1-like *G1P[8]* which infected 14% of RVA patients in Vietnam was reported to be

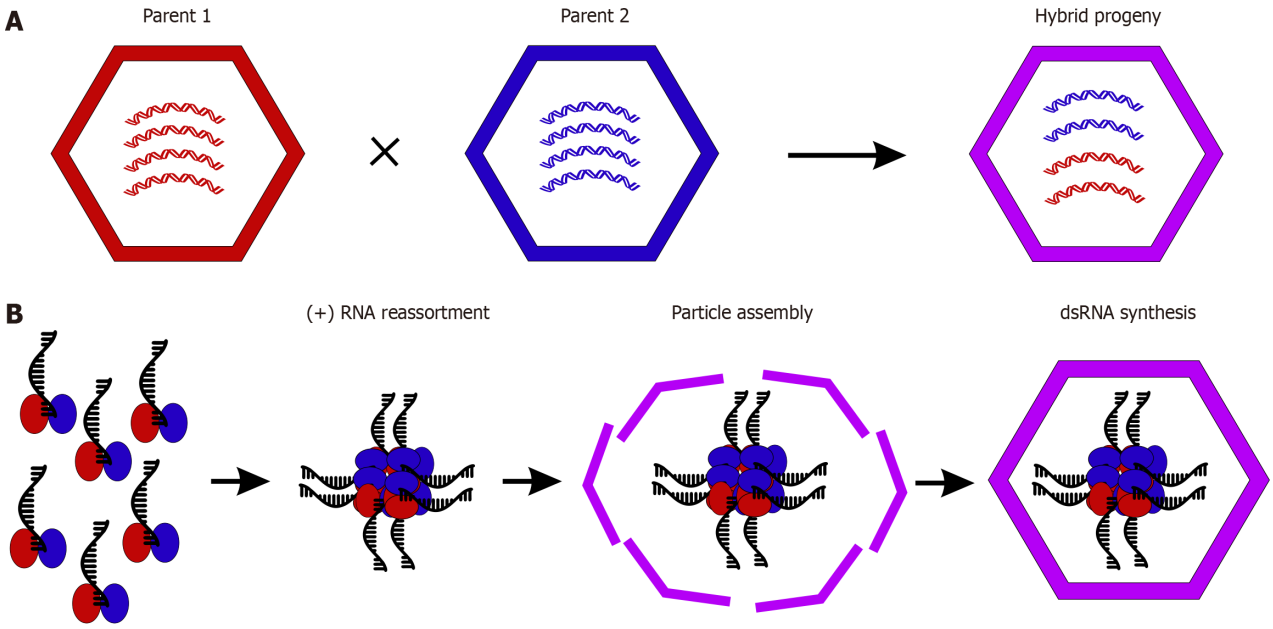

Figure 2 Reassortment mechanism of rotaviruses group A (RVA). A: Diagrammatic representation of the emergence of a novel reassortant strain with genes derived from two parental strains; B: Reassortment in RVA begins with the synthesis of positive-sense RNA. A total of newly-synthesized 11 positive segments of RNA will form a complex of supramolecular structures and be immediately assembled by virion particles that grow through the encapsidation process. The polymerase enzyme then converts positive RNA into dsRNA shortly after the encapsidation process.

rotavirus strains that evolved through an intergenogroup reassortment mechanism. *G2P[4]*, a local RVA strain in Vietnam, obtained the VP7 gene from a strain similar to *G1P[8]* and the VP4 gene of a strain similar to *G3P[8]* circulating in China. However, this double-gene reassortment of the RVA *G1P[8]* did not cause more severe diseases than the original *G1P[8]* strain[42].

Not only in Asia, RVA strain DS-like *G1P[8]* double-gene reassortant is also found in various other continents, such as Europe, Africa, Australia, and America[8]. RVA strain DS-1-like *G1P[8]* in Brazil was reported to have a phylogenetic similarity with strain DS-1-like *G1P[8]* in Asia and has a distant relationship with strains found in Africa. Thus, RVA strain DS-1-like *G1P[8]* in Brazil may have originated in Asia. Further sequence analyses demonstrated that RVA strain DS-1-like *G1P[8]* in Brazil also has a close relationship with the equine-like strain DS-1-like G3P[8] which indicates that they have the same origin[12].

Another DS-1-like intergenogroup reassortant of RVA strains has also been reported, such as DS-1-like *G8P[8]* in Thailand which is known to evolve through multiple reassortment mechanisms. The 11 genes were derived from the rotavirus strain of DS-1-like, bovine, bovine-like human, human, and locally circulating DS-1-like *G2P[4]*[8]. This shows that the evolution of RVA is not only able to take place through intragenogroup reassortment, but also through intergenogroup reassortment[8,12]. The emergence of RVA which evolved through the intergenogroup reassortment mechanism is capable of causing outbreaks, as occurred in Singapore in 2016 where the reassortant intergenogroup DS-1-like *G8P[8]* was found to be the main cause of gastroenteritis outbreaks[10].

The evolution of the RVA G12 strain was also reported. This G12 strain was initially very rare until 2008, but experienced a significant increase thereafter[9]. RVA strain G12 is the dominant strain in neonatal infection, for example in India, where the prevalence is about 24.31% of the neonates, without showing any symptoms[43]. This is similar to what reported in Spain, where the G12 strain was first detected in 2004, but went undetected again 4 years later. The epidemic of the Wa-like *G12P[8]* strain in Spain, particularly in the province of Gipuzkoa, occurred from early 2010 to 2018 and was able to exceed the number of cases of the G1 strains which had become the dominant strain from 1989 to 2009. These cases could be affected either by the migration of G12 strains from other countries to Spain which eventually caused epidemics, or by the local evolution of the G12 strains. This indicates the possibility of a reassortment event of G12 circulating in various countries and making contact with various other *RVA* strains causing the emergence of new *RVA* strains[9].

Point mutations (genetic drift)

In addition to the reassortment event of G12, the local evolution of this G12 strain also indicates the possible influence of other factors, such as interspecies recombination and accumulation of point mutations[44]. Generally, in RNA viruses, accumulation of point mutations is caused by their error-prone RdRp enzymatic activity, resulting in an estimated mutation rate (1.0×10^{-3} to 1.0×10^{-6} base substitution/site/year)[45]. Meanwhile, recent studies reported that intersegment recombination did not affect the long-term evolution of *RVA*[46]. The evolution of G12 strain was also found in Africa, where the African G12 rotaviruses which belongs to line III rotavirus has evolved and produced 2 sublines, namely the West African G12 rotavirus group and the South African G12 rotavirus group. This shows that the evolution of rotavirus can also be influenced by geographical location, where the topographical structure can be a barrier

to the movement of human populations, thus triggering genetic diversification in rotaviruses. The high genetic diversification and evolution of rotaviruses are due to the tendency for molecular evolution to occur due to a stable mutation rate, which ranges from 1.201×10^{-3} to 2.198×10^{-3} base substitution/site/year[44].

The effect of mutations on the evolution of rotavirus A was also reported in the RVA strain *G4P[8]* in Italy. This random point mutation in RVA strain *G4P[8]* led to the evolution of the strain into 3 circulating lines simultaneously. Apart from the effect of point mutations, the 3-line evolution of the *G4P[8]* strain was also influenced by reassortment2, particularly in the VP4, VP6, and NSP4 genes. This shows that the evolution of rotaviruses is not only influenced by genetic shifting due to reassortment mechanisms, but is also influenced by genetic drift events due to random point mutations[47].

P[II] genogroups such as *P[4]*, *P[6]*, and *P[8]* in human rotavirus are the most dominant rotavirus genotypes infecting humans. This dominance is influenced by the infectious ability of this genogroup which has higher specificity to the host as compared to other rotavirus genogroups (*P[I,III-V]*)[15]. The human rotavirus genogroup *P[II]* can infect host cells by binding to two types of glycans, mucin core and type 1 histo-blood group antigen (HBGA), in addition to binding to GlcNac as a ligand binding center. The interaction with these two types of glycans is not shared by other genogroups. *P[I]* genogroup that infects animals only has a binding site with the GlcNac ligand. This indicates that the rotavirus genogroup *P[I]* is the ancestor of the rotavirus genogroup *P[II]*. It is characterized by the *P[II]* that retains its binding site with the GlcNac ligand but has two additional glycan ligands which enable the infection to spread to both animals and humans. This also explains the existence of zoonotic transmission events in rotaviruses. Changes in the binding site with the glycan residue are able to trigger the evolution of the rotavirus genogroup *P[II]* so that it has a dominant ability to infect various types of animal and human hosts[16].

DRIVING FORCES OF ROTAVIRUS EVOLUTION

The key driving force behind the evolution and emergence or re-emergence of pathogenic viruses is the intricate 'host-pathogen-environment' relationship[2]. Therein, the relative importance of zoonosis is a function of the prevalence of reservoir animal species and the probability of close contact (direct or indirect) with the susceptible hosts. In addition, because RNA viruses are known to incorporate drastic mutations in their genomes, their new strains or genotypes can spread to different geographical regions with immunologically naive populations. It is also quite possible that such newly human-adapted viral strains could circulate asymptomatically and remain undetected until they manifest clinically. Nonetheless, to understand evolutionary dynamics as well as to accurately predict such epidemics or pandemics, few well-known computational and mathematical models have been developed[2].

Similar to other pathogenic RNA viruses, the incidence of rotaviruses in various countries which shows genotypic differences fluctuating over time has also shown its dynamic evolution. The evolution of rotavirus is influenced by a number of driving factors, such as vaccine introduction, the host immune responses, and antiviral drugs[17,21,39,40,42,43].

Vaccine introduction

The introduction of rotavirus vaccine has an important role in reducing the incidence of pediatric gastroenetritis. The national immunization program for rotavirus vaccine has been implemented in 123 countries as of January 2023. In addition, twelve countries have planned to introduce rotavirus vaccine for the national immunization program[48]. The vaccines can significantly reduce the morbidity and mortality of children under-five due to acute diarrhea[49]. There are two types of live attenuated, oral rotavirus vaccines that have received WHO approval and have been used in more than 100 countries: Rotarix (GSK Biologics) and RotaTeq (Merck and Co.). Rotarix is a monovalent rotavirus vaccine containing human rotavirus strain *G1P[8]*, while RotaTeq is a pentavalent rotavirus vaccine containing human rotavirus reassortant strains G1, G2, G3, G4, and *P[8]*[4]. In high-mortality countries (*i.e.*, Africa and Asia), Rotarix and RotaTeq vaccines have equal vaccine efficacy against severe rotavirus gastroenteritis with 57% during one-year after vaccination. Meanwhile, after two-years follow-up, the efficacy of Rotarix and RotaTeq vaccines are 29% and 44%, respectively[50]. Safety data obtained from the clinical trials showed that rotavirus vaccines are well tolerated. A Cochrane systematic review of available evidence indicated no increase in serious adverse events associated with Rotarix and RotaTeq vaccines[51]. There is no increased risk of intussusception among vaccinated children, although safety surveillance should be continuously conducted[51]. In addition, epidemiological and immunological studies show a possible association between rotavirus infection and autoimmune diseases, most commonly celiac disease. Thus, the rotavirus vaccines should be able to decrease this autoimmune disease-associated rotavirus infection, although it is still controversial[52,53].

In addition to these two vaccine being used globally, there are several rotavirus vaccines that have been approved in specific countries, such as Rotasiil and Rotavac (India), Lanzhou Lamb Rotavirus (China), and Rotavin-M1 (Vietnam)[43,54-56]. Rotasiil contains human reassortant rotavirus strains G1, G2, G3, G4, and G9, while Rotavac contains *G9P[11]* strains[54,56]. LLR and Rotavin-M1 are monovalent rotavirus vaccines for the animal rotavirus strain *G10P[12]* and the human rotavirus strain *G1P[8]*, respectively[43,55]. Within one-year follow-up, the efficacy of Rotavac and Rotasiil vaccines against severe rotavirus gastroenteritis are 57% and 48% respectively. While, two years after vaccination, the efficacy of Rotavac and Rotasiil vaccines decline to 54% and 44%, respectively[50]. Although it has been implemented by several countries, these four types of vaccines are still in the WHO pre-qualification stage[4].

Notably, while vaccines have an important role in preventing rotavirus infection, they are also an important driver of rotavirus evolution. The evolution of rotavirus can be characterized by the emergence of an unusual strain of RVA which is characterized by the ineffectiveness of the introduced vaccine in preventing infection[13]. The ineffectiveness of this

vaccine causes children who have been fully or partially vaccinated to remain susceptible to the symptomatic rotavirus infection[57]. The introduction of RotaTeq and Rotarix in Korea in 2007 and 2008, respectively, was associated with an alteration of dominant genotype constellation of genogroup 1 to 2. Prior to the introduction of the vaccines, RVA strain *G1P[8]* was the dominant RVA genotype circulating in Korea. However, after the introduction of the vaccine, *G2P[4]* which was very rarely detected before the introduction of the vaccine became the dominantly circulating RVA strain. Subsequent genetic analysis showed that the *G2P[4]* genotype which emerged after the introduction of the vaccine in Korea in 2007, underwent multiple interspecies reassortment. This *G2P[4]* reassortant strain has a constellation of DS-1-like genotypes and acquired NSP4 genes from cattle and buffalo, and VP1 and VP3 genes from goats. In addition to being a reassortment, a mutation in the VP7 gene also potentially causes the RotaTeq or Rotarix vaccines to be less effective[58].

Interspecies reassortment following the introduction of the vaccines was also reported in Belgium, where RVA strain DS-1-like *G2P[4]* acquired a bovine-like NSP4 gene segment of animal origin. In addition, there are six other gene segments that are also involved in interspecies reassortment of this *G2P[4]* strain, *i.e.* VP6, VP1-3, NSP2, NSP4 and NSP5 genes, where two NSP4 clusters and one VP3 cluster tend to be preserved in human-to-human viral transmission. Interspecies reassortment causes dead-end infection since the majority of animal-human interspecies reassortants rarely show human-to-human transmission. Although the main factor in the emergence of RVA *G2P[4]* strain in Belgium was the selection pressure from the vaccine introduction, another influencing factor was the migration of *G2P[4]* from other regions, which was supported by the presence of a bovine-like NSP4 gene segment that tends to be retained in human-to-human transmission[59].

In addition to interspecies reassortment, intragenogroup reassortment of RVA strain Wa-like *G1P[8]* was also reported in Belgium after the introduction of the Rotarix vaccine. Reassortment can also occur between circulating rotavirus strains and the introduced vaccine strains. The evolution of *G1P[8]* is also observed from the notable difference between the circulating *G1P[8]* gene segment after vaccine introduction and the Rotarix vaccine strains, such as VP6, VP2, and NSP2 genes. This difference may be due to the accumulation of point mutations that make Rotarix vaccine less effective in preventing the infection due to circulating rotavirus strain *G1P[8]* [60].

Similar phenomena were observed following rotavirus vaccine introduction in Venezuela, where there was a change in dominance from *G1P[8]* to *G2P[4]*, followed by the emergence of various unusual genotype combinations, such as *G8P[14]*, *G1P[4]*, *G4P[4]*, and *G8P[4]*. Mutations in VP7 and reassortment are also known to be essential factors driving the evolution of the rotaviruses. A mutation in the VP7 neutralization domain in the form of a D96N substitution is known to have an important role in the emergence and dominance of the G2 rotavirus strain. However, the dominance of *G2P[4]* in Venezuela only lasted for one year, with the emergence and re-dominance of the *G1P[8]*. This could be due to differences in the viral fitness between susceptible and resistant hosts, thus giving rise to dynamics between rotavirus strains. The re-emerging *G1P[8]* is known to have mutations in VP7, resulting in escape of the antibodies induced by the rotavirus vaccine[14]. The predominance of *G2P[4]* strain after vaccine introduction was also found in Botswana[61].

Generally, the introduction of vaccines does not necessarily affect the changes in the dominance of circulating strains in a country. The introduction of the vaccine in east and south African countries did not result in any changes in the circulating strains. RVA strain *G1P[8]* was the dominant circulating strain, either before or after vaccination. However, some unusual combinations of RVA genotypes were found in low frequency, such as *G1P[4]*, *G2P[8]*, *G9P[4]*, and *G12P[4]*. These strains arise due to intragenogroup or intergenogroup reassortment mechanisms[62]. The introduction of other rotavirus vaccines, such as Rotavac in India, has also led to the emergence of unusual combinations of RVA genotypes, such as *G9P[4]*, *G2P[6]*, *G2P[8]*, *G12P[4]*, and *G1P[11]*[63]. Furthermore, the introduction of the rotavirus vaccine was able to significantly reduce the incidence of acute gastroenteritis due to rotavirus infection in pediatric population (< 5 years). However, other viral infections such as norovirus pose new problems because they are the main disease agent of acute gastroenteritis with higher severity after rotavirus vaccine introduction[64,65].

Host immune responses in rotavirus infection

Humans have innate and adaptive immune defense systems to fight microbial infections, inc2luding viruses. The host cells have a number of membrane-bound and cytoplasmic receptors that function to recognize viral-derived nucleic acids, including toll-like receptors and RIG-I-like receptors. The binding of the ligand-receptor will trigger the activation of a series of downstream signaling to increase the expression of pro-inflammatory cytokines and chemokinesto activate the antiviral response and provide danger signals to neighboring cells, generating antiviral states[66]. Interferons (IFNs) that play a role in the antiviral responses in the infected cells are type I and III IFNs. Type I IFN was found in high concentrations in the serum of rotavirus-infected hosts, while type III IFN had a more specific role in the antiviral response in the epithelial cells, including in the intestines, lungs, and skin. IFN signaling induces the expression of interferon-stimulated genes (ISGs) as an antiviral effector protein which functions to limit viral replication[67,68].

In addition to IFN-λ which belongs to type III IFN, interleukin 22 (IL-22) is also known to have an essential function in protecting the surface barrier of intestinal epithelial cells from rotavirus infection. IL-22 produced by innate lymphoid cell group 3 (ILC3) functions as an amplifier of IFN-λ. IL-22 and IFN-λ work in synergistic manner to prevent infection and viral replication. The presence of these two molecules is required for optimum activation of the STAT2 transcription factor[69]. Besides ILC3, macrophages are also known to play a role in producing cytokines after rotavirus infection, particularly IFN I and other antiviral cytokines[70]. Furthermore, microbiota that have immunomodulating and antiviral abilities such as *Bifidobacteria* are known to enhance the innate immune response to fight infection and rotavirus replication[71].

In addition to innate immunity, the adaptive immune response obtained following vaccination or natural infection also plays an important role in eliminating rotavirus infection[72]. The immunological defense mechanism against rotavirus has yet to be comprehensively elucidated up to this moment. However, it is known that IgA, neutralizing antibodies, and T cells have an important role in fighting rotavirus infection[73]. The essential role of IgA in defending rotavirus infection

was shown in mice models deficient of the IgA gene. When compared to the wild-type mice, $IgA^{-/-}$ mice had a considerable and significant delay in clearing rotavirus infection[74].

The B and T cells have important roles in preventing rotavirus infection and controlling rotavirus replication, respectively[17]. In addition to an increase in IgA, rotavirus infection or the introduction of vaccines can induce an increase in antibody-secreting B cells that reside in the intestines[75]. Rotavirus infection is able to induce a T-cell immune response in the serum of children under 5 years of age[73]. However, rotavirus has the ability to escape from the T cells through inhibition of the expression of MHC class I which is required by T cells to kill virus-infected cells[19].

Although the host has mechanisms to limit and prevent infection, rotaviruses have a number of strategies to evade the host's innate immune system in order to survive and reproduce. The existence of this immune system evasion strategy is the main key for rotavirus to be able to evolve and have high genetic diversity. There are three rotavirus proteins involved in the evasion mechanisms of the host innate immune system, i.e. NSP1, VP3, and NSP3 (Figure 3)[18].

NSP1 can trigger rotavirus evasion of the host innate immune response in two ways: proteasome-dependent or proteasome-independent. The proteasome-dependent innate immune response evasion strategy in animal rotavirus strains occurs *via* degradation of IRF by the NSP1-induced proteasome. In human and porcine rotavirus strains, it occurs *via* degradation of β-TrCP by the NSP1-induced proteasome. In addition, NSP1 is also involved in the proteasome-mediated degradation of a number of other proteins involved in the antiviral immune response, for example the TRAF protein family involved in the NFκB pathway. Proteasome-independent mechanisms of NSP1 induces the blocking of IRF3 transcriptional activity or preventing RIG1 and MAVS signaling which leads to prevention of IFN and ISG activation in the host cells. Prevention of ISG transcription in the host cells by rotavirus also occurs through the blocking mechanism of STAT1 phosphorylation and inhibition of the translocation of STAT1 and STAT2 to the nucleus[76].

The mechanism of evading the host innate immune response by rotavirus VP3 protein occurs through the degradation process of 2',5'-oligoadenylate (2-5A) molecules which are known to form complexes with RNase L that are able to degrade rotavirus dsRNA. VP3 is able to degrade 2-5A because it has 2',5'-phosphodiesterase activity in its C-terminal domain, thus preventing the activation of RNase L. Meanwhile, NSP3 induces evasion of the host innate immune response by interacting with eIF4G and is involved in relocation of poly(A)-binding protein (PABP) that leads to inhibition of host cell mRNA translation. NSP3 has a higher affinity for eIF4G than PABP and is an RNA-binding protein specific for rotavirus mRNAs. Saturation of the translation initiation machinery with viral mRNAs possibly leads to cellular protein synthesis inhibition, including proteins involved in the host cell antiviral immune response[77].

Antiviral drugs

In contrast to the WHO approved rotavirus vaccines being used in > 100 countries, there is no specific anti-rotavirus drugs available to date. Currently however, the most effective treatment of rotaviral acute diarrhea is through palliative or supportive therapy in the form of rehydration therapy, by restoring the lost body fluids to prevent dehydration. However, several anti-rotavirus drugs have been extensively studied for their potential in inhibiting rotavirus infection [23]. Furthermore, one thing that should be considered is the possibility of the emergence of viral strain that resistant to the newly developed antiviral drugs. Anti-HIV drug resistance is one of the important examples of the emergence of viral strain that evolved after the introduction of the antiviral drugs for viral infection treatment[78]. Therefore, care should be taken starting from the development of the anti-rotavirus drugs.

One of the anti-rotavirus drugs that are still in the early research stages is ursolic acid (UA). UA is a pentacyclic triterpenoid which is known to have antiviral activity against rotavirus infection. UA is able to significantly reduce the amount of rotavirus viral protein and inhibit the growth of infective rotavirus progeny in the initial cycle of infection. The decrease in the number of viral proteins and infective progenies could be due to the decrease in the intraluminal calcium ion content of the endoplasmic reticulum. This will result in folding errors in VP7 protein and imperfect glycosylation of NSP4 and VP7 proteins which leads to failure of rotavirus maturation. However, at the end of the rotavirus cycle, UA did not show any significant effect on the growth pattern of rotavirus. This could be due to a number of rotavirus progenies that have been generated before the administration of UA[20]. In addition, pyrrole derivatives such as 'pyrrolo [2,3-d] pyrimidine' and 'pyrrolo [3,2-e][1,2,4] triazolo [4,3-c] pyrimidine' are also alternative antiviral drugs and have a high activity against rotavirus infection[23].

Not only synthetic drugs, but other approaches have also been done to prevent rotavirus infection by utilizing the microbiota. Segmented filamentous bacteria (SFB) inoculated in the intestines of mice was able to make the mice resistant to rotavirus infection. The mechanism that causes this to occur has not been able to be explained comprehensively. However, SFBs are capable of causing resistance to rotavirus infection by blocking an important component of rotavirus that is used to bind to host cells, and by inducing the production of IL-22 which influences enterocyte proliferation and translocation[21].

Interactions with other enteric viruses

The intestinal environment also harbors a eukaryotic virome of significant human viruses, such as RV, norovirus, astrovirus, and enteric adenoviruses, responsible for viral gastroenteritis. These viruses replicate within a spectrum of cell types, including enterocytes, lymphocytes and myeloid cells[79]. Rotavirus and norovirus recognize different HBGAs as initial receptors for attachments[80]. In pediatric patients, it was observed that viral shedding was observed significantly longer for norovirus than rotavirus[81]. Importantly, in nations where RV vaccination initiatives have been implemented, the incidence of rotavirus etiology has significantly declined. However, norovirus comparatively continues to emerge as the predominant pathogen of pediatric gastroenteritis[82]. In an Indian three-year hospital-based surveillance study of acute gastroenteritis, norovirus positivity in rotavirus -vaccinated and unvaccinated children were 16.3% and 12%, respectively[83].

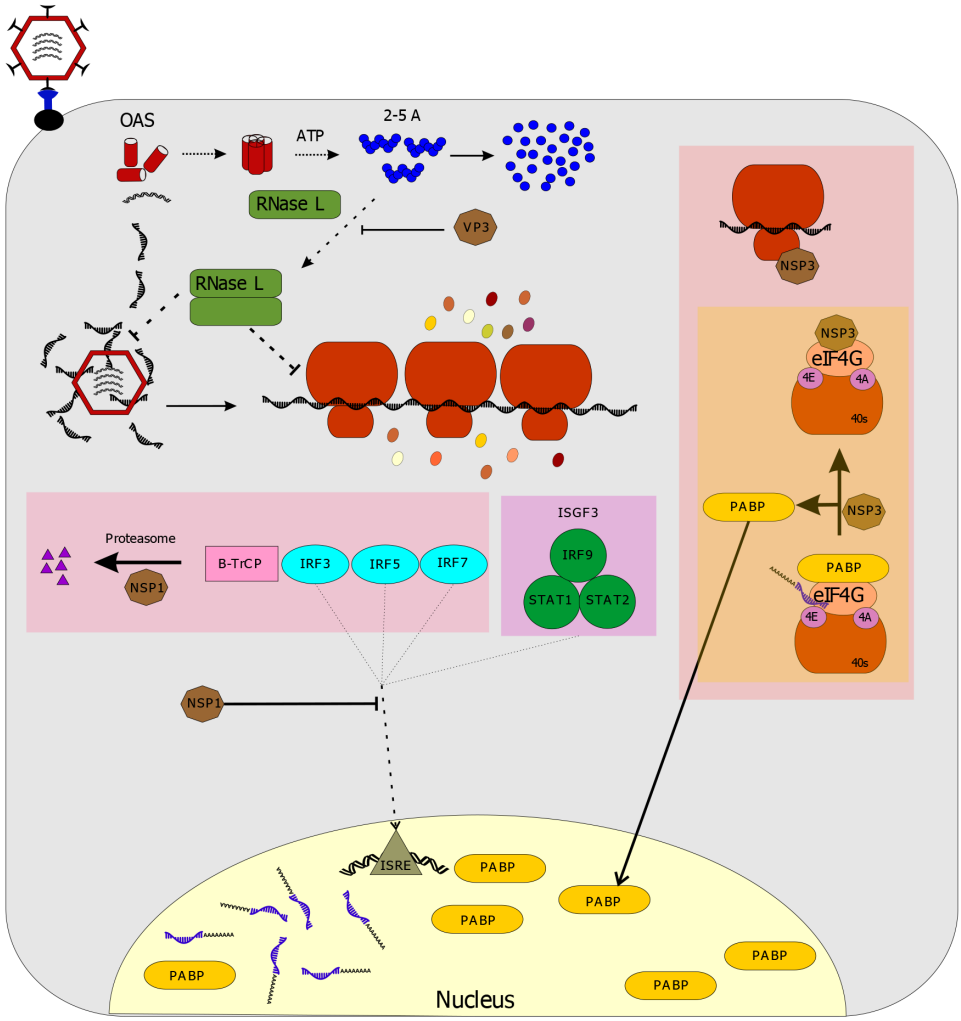

Figure 3 Cartoon representation of Rotavirus strategy to evade the host innate immune system. OAS: Oligoadenylate synthase; β-TrCP: Beta-transducin repeats-containing proteins; IRF: Interferon regulatory factor; STAT: Signal transducer and activator of transcription; PABP: Poly(A) binding protein; ISRE: Interferon-stimulated response element; eIF4G: Eukaryotic translation initiation factor 4G.

Further, enteric adenoviruses replicate efficiently in human organoid models, particularly goblet cells[84]. In children with acute gastroenteritis, a high adenoviral load was noted during the first few days of infection, which rapidly declined [81]. In contrast to rotavirus, the prevalence of adenovirus infections was relatively low[85,86]. During the COVID-19 pandemic, the incidence of adenovirus infection was also lower as compared to pre-pandemic period[87]. In one study, it was reported that adenovirus was the most common diarrheal pathogen following rotavirus vaccine introduction in India [88]. Adenovirus infection was also higher in rotavirus-vaccinated than rotavirus-unvaccinated children in Venezuela [89]. All these findings suggest a dynamic coevolution of enteric viruses, especially after the introduction of nationwide rotavirus vaccination.

CONCLUSION

Rotaviruses are the causative agent of acute diarrheal disease in pediatric population. Similar to other RNA viruses, rotaviruses continuously evolve as indicated by the temporal and geographical fluctuation of the circulating strains as well as the emergence of unprecedented new strains. The antigenic shift and antigenic drift may result in more virulent rotavirus strains compared to the previously known strains. However, it is unlikely that the novel strains would have a higher viral fitness (replication and transmission fitness) than the currently circulating strains, given that the latter have been adapting for many centuries in the human population. Presumably, hitherto there is an undiscovered dynamic pool of human-adapting viruses of these while some virus species tend to become extinct, others continue to evolve in their natural hosts.

Rotavirus evolution is driven by several factors, most importantly vaccines and the host immune responses. However, other factors are also known to have an impact on rotavirus evolution, including adaptation to the host species. The evolution of rotavirus can be characterized by the emergence of unusual strains of RVA which potentially reduced the effectiveness of the introduced vaccines in preventing infection. The emergence of these novel strains may significantly

impact global health, particularly in children < 5 years of age. Consequently, continued surveillance of rotaviruses circulating worldwide both in human and animal species should be intensified to monitor the novel strains as well as to evaluate vaccine effectiveness. Thus, understanding the evolutionary dynamics of emerging rotavirus strains and their associated driving factors is essential to develop effective preventive and therapeutic measures.

FOOTNOTES

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Country of origin: Saudi Arabia

ORCID number: Mohamad Saifudin Hakim 0000-0001-8341-461X; Mohammad Khalid Parvez 0000-0002-7154-9151.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Lab results of COVID-19 patients: Omicron *vs* delta variants

Dana Avraham, Amir Herman, Gal Shaham, Arkady Shklyar, Elina Sulim, Maria Oulianski

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Dana Avraham, Amir Herman, Maria Oulianski, Orthopedic Department, Kaplan Medical Center, Rehovot 7661041, Israel

Gal Shaham, Department of Medicine, Lithuanian University of Health Sciences, Kaunas 44307, Lithuania

Arkady Shklyar, Elina Sulim, Emergency Department, Kaplan Medical Center, Rehovot 7661041, Israel

Corresponding author: Dana Avraham, MD, Doctor, Orthopedic Department, Kaplan Medical Center, Pasternak 1, Rehovot 7661041, Israel. danaav7111@gmail.com

Abstract

BACKGROUND

The coronavirus disease 2019 (COVID-19) virus has been a world-known pandemic since February 2020. Multiple variances had been established; the most common variants in Israel were omicron and delta.

AIM

To analyze and compare laboratory values in the "omicron" and "delta" variants of the coronavirus by conducting follow-up examinations and laboratory audits on COVID-19 patients admitted to our institution.

METHODS

A retrospective study, two groups, 50 patients in each group. Patients examined positive for COVID-19 were divided into groups according to the common variant at the given time. We reviewed demographic data and laboratory results such as complete blood count and full chemistry, including electrolytes and coagulation parameters.

RESULTS

The mean age was 52%, 66.53 ± 21.7 were female. No significance was found comparing laboratory results in the following disciplines: Blood count, hemoglobin, and lymphocytes (P = 0.41, P = 0.87, P = 0.97). Omicron and delta variants have higher neutrophil counts, though they are not significantly different (P = 0.38). Coagulation tests: Activated paritial thromoplastin test and international normalized ratio (P = 0.72, P = 0.68). We found no significance of abnormality for all electrolytes.

CONCLUSION

The study compares laboratory results of blood tests between two variants of the

COVID-19 virus – omicron and delta. We found no significance between the variants. Our results show the need for further research with larger data as well as the need to compare all COVID-19 variants.

Key Words: COVID-19; Coronavirus; Omicron variant; Delta variant

Core Tip: We reviewed lab results of patient positive for coronavirus disease 2019 (COVID-19) during the periods of omicron and delta variants in Israel. retrospective study of patient 18-99 YO excluding pregnant and oncologic patients. The neutrophil index increased above the normal level. There was no difference between the variants in the other count parameters (hemoglobin and white blood cell count) and coagulation functions (activated paritial thromoplastin test, international normalized ratio) and electrolytes (sodium, chloride, phosphorus, and albumin) had no significant variances or deviations from the acceptable norma. hypokalemia was measured in 62% of all COVID-19 patients.

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INTRODUCTION

The first case of the coronavirus [(coronavirus disease 2019, COVID-19), severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)] was announced in December 2019 in Wuhan, China[1,2]. In February 2020, the first coronavirus case was officially detected in Israel[3]. Since its discovery, it has been extensively researched. Furthermore, there is a tendency for variants to mutate over time[4-6].

The virus causes various clinical symptoms, the most common of which are respiratory symptoms, muscle and joint pain, loss of appetite, and loss of smell. The elderly population has shown the highest risk for morbidity and mortality due to COVID-19. Patients over the age of 60 with multiple comorbidities have been shown to be at an incredibly high risk for severe acute respiratory distress syndrome, with mortality rates reaching up to 45% in comparison to the young and relatively healthy population -7.8%[7-9]. Although some infected individuals are asymptomatic, they will still carry and spread the virus[10].

At the beginning of 2022, Botswana and South Africa discovered the "omicron" variant (B.1.1.529). It raised concerns due to a large number of mutations in the spike protein, possibly impacting transmissibility and immune response. The delta variant (B.1.617.2) was first identified in India in late 2020 and became a predominant strain globally. It was associated with increased transmissibility compared to earlier variants. Studies suggested an increased risk of hospitalization with the delta variant.

The omicron variant showed some similarity to the "delta" variant for mutations in Q498R and N501Y, strengthening its ability to bind to the ACE2 protein, the most spreadable variant. In addition, the "omicron" has a wide-ranging adhesive ability due to a mutation in H655Y, N679K, and P681H in the cutting/cleavage region of S1-S2 Furin. Moreover, there is evidence of multiple additional mutations of the "omicron" variant[11,12].

Patients who attend the ED (emergency department) can be divided into two groups: the first one with flu-like symptoms and general symptoms, and the second group are patients who had a positive COVID-19 exam at home and then attended the ED. All patients who are admitted to the ED due to COVID-19 symptoms are routinely subjected to various laboratory tests. In many cases, making the appropriate decisions during the initial treatment phase can significantly impact disease progression. Hospitalization of COVID-19 patients is approved in cases where the patient presents with an "in hospital" positive COVID-19 exam and respiratory symptoms.

Today, an antigen test is performed for the initial mapping of patients. However, the question arises: Are there any changes in laboratory data parameters among coronavirus patients who carry the different variants? There is no consistent information on laboratory data in the preliminary phase when a patient is admitted to the ED. Numerous studies have analyzed laboratory results of patients receiving intensive care during hospitalization and general laboratory values for COVID-19 patients, but the difference between various variants has not been examined.

According to the WHO organization, in November 2022, the main variant in Israel was the "omicron," with the first case being observed in November 2021[12-15]. Meanwhile, the first "delta" variant case was discovered in Israel in May 2021. Figure 1 illustrates the month-by-month distribution of the various variants in Israel. It is essential to note that there is a substantial variance between countries worldwide and the presence of a wide range of varieties at any given time [16]. Figure 2 display the Potassium levels of patients with Omicaron and Delta variants.

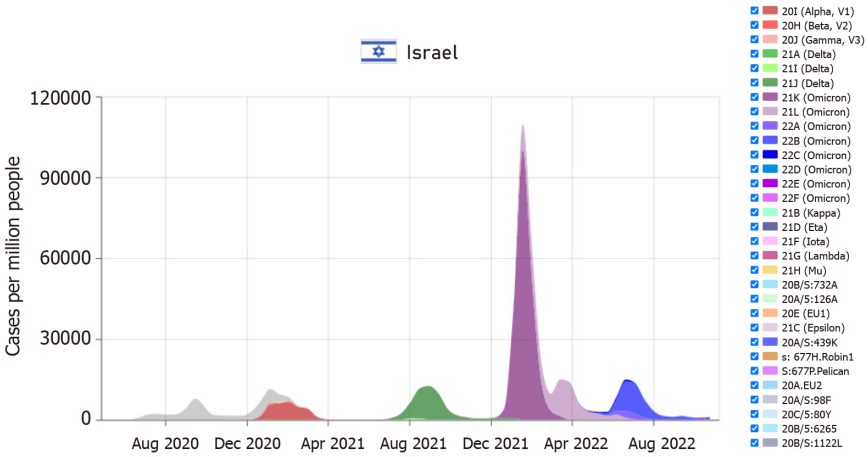

Figure 1 Distribution of the corona virus and the different variants by month in Israel[13].

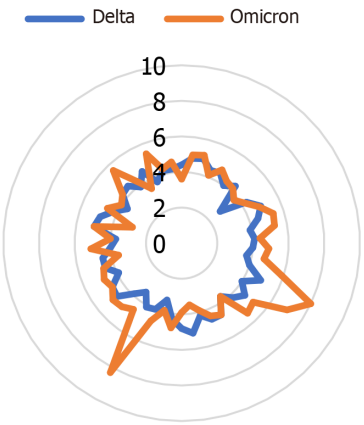

Figure 2 Scattering graph of laboratory results for potassium. It can be noticed that the majority of the potassium readings during the "delta" variant period are lower than those during the "omicron" variant era.

MATERIALS AND METHODS

At the beginning of 2020, many patients infected with the COVID-19 virus were admitted to our institution. Like all other patients who attend the internal medicine ED, these patients undergo a series of tests for confirmation and assortment of their health status before their hospitalization to the specific internal world or intensive care departments. These exams include a chest X-ray, ECG, and preliminary laboratory tests.

A retrospective cohort study was conducted on patients diagnosed with COVID-19 in our ED. Findings were reported according to the STrengthening the Reporting of OBservational studies in Epidemiology checklist for retrospective cohort studies, and the local ethics committee approved the study. Data from electronic patient files were retrospectively extracted from the medical records of patients with a diagnosis of the COVID-19 virus who were admitted to the ED of a tertiary medical center in the center region of Israel between August 2021 and April 2022. The range of dates was chosen to include the "delta" period from 08/02/2021 to 11/22/2021 and the "omicron" period from 01/15/2022 to 04/04/2022. Patients included in the study were adults, 18 to 99 years old, who had been hospitalized due to a coronavirus diagnosis—a positive test at admission to the ED, and they have not tested positive for coronavirus in the past, with an exemption for self home exam before attending the hospital. Concurrently, a comprehensive review of dates where the delta and omicron variants exhibited 100% dispersion. The study groups were divided according to the patient's hospitalization dates, corresponding to a specific variant, consisting of 50 patients. Each patient tested positive in at least two "COVID-19" tests that have been done in our hospital. The first positive test was from the ED, and the second was in the internal medicine department upon admission to the department. Additionally, laboratory assessments include blood chemistry, electrolytes, complete blood count, and coagulation blood tests.

Exclusion criteria included pregnancy, young age (under 18), hospitalizations for other diseases/conditions or trauma injuries unrelated to the coronavirus, and underlying diseases that could bias laboratory values and be confounding, such as chronic white blood cell diseases.

The study aims to analyze and compare laboratory values in the "omicron" and "delta" variants of the coronavirus by conducting follow-up examinations and laboratory audits on COVID-19 patients admitted to our institution.

The data was processed using IBM SPSS Statistics software for Windows (version 25.0, Armonk, NY, United States). Charts and curves were drawn using Microsoft Excel 365 (version 2101, Microsoft Corp., Redmond, WA, United States). Continuous measures were assessed for distribution and were presented as mean and standard deviation. Categorical data were presented as prevalence and percentages. Comparisons involving categorical variables were performed using a student's *t*-test. Furthermore, a multivariate analysis was conducted to examine the clinical and demographic data in relation to various laboratory results.

RESULTS

This study included 100 patients, 50 in the "delta" group and 50 in the "omicron" group. The average age was 66.53 ± 21.7. The majority of the patients were females (52%). No statistically significant differences were identified in age, gender, and comorbidity between the "delta" and the "omicron" variants ($P = 0.9$, $P = 0.23$, respectively) (Table 1).

Laboratory analysis

No significant differences were found in the index of complete blood count: white count - without leukocytosis, hemoglobin, and lymphocytes, indicators within the normal range ($P = 0.97$, $P = 0.87$, $P = 0.41$ respectively), the neutrophil count was slightly above the norm, but without a statistically significant difference between the variants ($P = 0.38$). Coagulation functions did not show significant differences in international normalized ratio (INR) and activated paritial thromoplastin test (APTT) parameters ($P = 0.72$ and $P = 0.68$, respectively). There were no significant findings for electrolytes in any of the following: sodium, potassium, chloride, phosphorus, and albumin. None of the indicators deviate from the norm. The closest index to significance is quasi-significant potassium $P = 0.09$ (Table 2).

DISCUSSION

COVID-19 significantly impacts hospital activity, commencing with a change in the ER and other departments' work structure and the allocation of personnel in all medical and paramedical sectors[17,18]. Today, patients frequently present to our medical Center following a home coronavirus diagnosis and after a new onset of specific symptoms associated with the virus. Over the last two years, many investigations have been conducted to identify and comprehend the virus, prevent its spread, and develop a cure[19].

In Israel, a significant percentage of the population has received multiple vaccine doses, which have been shown to affect the symptoms presented by patients and the severity of the disease's clinical manifestation[20-22]. Nevertheless, we continue to see some ER admissions frequently represented in waves. Over the last two years, the coronavirus has continued to evolve, and new variants were discovered in our districts. Our study examined the most common variants in Israel, "omicron" and "delta". We compared the omicron variant with the delta variant. The two study groups have similar demographic data regarding age, gender, and past illnesses. The following basic laboratory data were compared: complete blood count, coagulation, and blood chemistry. The study by Qin *et al*[23] (2020) found that lymphopenia is one of the leading indicators of COVID disease, primarily when the disease is defined as severe. This tendency and the neutrophil-lymphocyte ratio were also observed in Israel. In our study, there was no significant statistical difference between the two variants and no deviation from the accepted standard[24]. The neutrophil index increased above the normal level in our study, consistent with past research[25]. There was no difference between the variants in the other count parameters (hemoglobin and white blood cell count) and no significant variances or deviations from the acceptable norma. Coagulation functions (APTT, INR) did not differ significantly among the variants. According to the literature, hypokalemia was measured in 62% of all COVID-19 patients infected with the initial variants[26]. In our research, despite this index being the closest to significance among the electrolytes, there was no significant difference between the variants. The other electrolytes (sodium, chloride, phosphorus, and albumin) showed no significance or deviation from the accepted norma.

There are various possible limitations to this study. First, this study only included a single tertiary hospital, which cannot give insight into other medical institutes in our country. The second limitation is the small number of patients who participated in the study; a larger sample group is needed in future studies. Our study did not discover any significant differences in comparing accepted laboratory results during the initial testing phase within the ER setting for the two most recent coronavirus variants in Israel. We could not find a similar comparative study for the "omicron" and "delta" variants in the literature review.

CONCLUSION

While this article provides valuable insights into specific laboratory aspects of delta and omicron variants of COVID-19, it is essential to acknowledge its limitations in failing to compare clinical parameters of the virus variants. Future research endeavors should consider encompassing diverse variants to ensure a more nuanced and systemic approach to addressing the challenges posed by COVID-19.

Table 1 Demographic information

	All	Delta	Omicron	*P* value
Age (SD)	66.53 (21.7)	66.23 (23.03)	66.80 (20.51)	0.90
Gender-female (%)	52%	58% (29)	46% (23)	0.23
Mortality (%)	22%			

Table 2 Comparison between "delta" and "omicron" of laboratory parameters, including complete blood count, blood chemistry, and coagulation factors

	All	Delta	Omicron	*P* value
WBC (SD)	9.58 (5.95)	10.07 (6.57)	9.09 (5.29)	0.41
Hb (SD)	12.64 (2.07)	12.67 (1.51	12.61 (2.52)	0.87
Neutrophils abs (SD)	7.91 (5.69)	8.41(6.31)	7.41 (5.01)	0.38
Lymphocytes (SD)	0.92 (0.66)	0.92 (0.78)	0.92 (0.52)	0.97
PT INR (SD)	1.04 (0.12)	1.04 (0.11)	1.03 (1.13)	0.68
APTT (SD)	27.42 (5.15)	27.24 (4.8)	27.64 (5.59)	0.72
Sodium (SD)	138.19 (5.47)	138.48 (4.99)	137.90 (5.96)	0.60
Potassium (SD)	4.53 (0.78)	4.38 (0.69)	4.68 (1.01)	0.09
Chloride (SD)	103.46 (6.13)	103.60 (4.93)	103.32 (7.20)	0.82
Phosphorus (SD)	3.67 (1.92)	3.56 (1.38)	3.86 (2.65)	0.55
Albumin (SD)	3.33 (0.60)	3.29 (0.58)	3.29 (0.58)	0.44

WBC: White blood cells; Hb: Hemoglobin; PT: Prothrombin time test; INR: International normalized ratio; APTT: Activated paritial thromoplastin test.

ARTICLE HIGHLIGHTS

Research background

The article provides a comprehensive overview of the timeline and global impact of the coronavirus disease 2019 (COVID-19) pandemic, emphasizing the emergence of variants such as delta and omicron. Noteworthy symptoms of COVID-19 and the heightened risk among the elderly population are highlighted, including severe acute respiratory distress syndrome risks. The delta variant, identified in India in late 2020, is characterized by increased transmissibility and a higher risk of hospitalization. The omicron variant, first detected in Botswana and South Africa in early 2022, raised concerns due to a significant number of spike protein mutations.

Research motivation

To shed light on the laboratory aspects of COVID-19 patients during the emergence of the delta and omicron variants.

Research objectives

To providing valuable insights into the initial testing phase within the emergency department setting.

Research methods

The study included 100 adult patients, 50 for each variant, with comprehensive laboratory assessments. Rigorous exclusion criteria were applied to ensure the focus on COVID-19-related factors.

Research results

No statistically significant differences were identified in age, gender, and comorbidities between delta and omicron groups. Laboratory analyses, including complete blood count, coagulation, and blood chemistry, revealed no significant variations between the two variants.

Research conclusions

COVID-19 continues to mutate and evolve with different variants emerging, Future research endeavors should consider encompassing diverse variants to ensure a more nuanced and systemic approach to addressing the challenges posed by COVID-19.

Research perspectives

Both omicron and delta variants show high infection rates, similar laboratory results, but clinical evaluation should be conducted to determine the true similarity of those variants.

FOOTNOTES

Author contributions: Avraham D and Oulianski M designed the research; Herman A and Shaham G performed the research; Shklyar A contributed new reagents/analytic tools; Oulianski M and Sulim A analyzed the data; Avraham D and Oulianski M wrote the paper.

Institutional review board statement: The study was reviewed and approved by the "Kaplan Medical Center" Helsinki Committee Institutional Review Board [(Approval No. 0018-22-KMC]).

Informed consent statement: According to the Helsinki Committee decision, our study does not require inform consent.

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Data sharing statement: The authors commit to making the data and materials underlying the findings of this medical article available upon reasonable request. Requests for data should be directed to [Dr. Dana Avraham at Danaav7111@gmail.com. The authors aim to facilitate transparency and reproducibility in scientific research and encourage collaboration within the scientific community. Access to the data will be provided in compliance with ethical standards and institutional regulations.

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ORIGINAL ARTICLE

Retrospective Study

Paradigm shift in transfusion practices during early COVID-19 pandemic: A single center retrospective study

Sumukh Arun Kumar, Sushmita Prabhu, Ankushi Sanghvi, Maya Gogtay, Mithil Gowda Suresh, Harshit Khosla, Yuvaraj Singh, Ajay Kumar Mishra, Susan George

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Sumukh Arun Kumar, Sushmita Prabhu, Ankushi Sanghvi, Mithil Gowda Suresh, Susan George, Department of Internal Medicine, Saint Vincent Hospital, Worcester, MA 01608, United States

Maya Gogtay, Department of Hematology and Oncology, University of Nebraska Medical Center, Omaha, NE 68198, United States

Harshit Khosla, Hospice and Palliative Care, UTHSC and McGovern School of Medicine, Texas, TX 77030, United States

Yuvaraj Singh, Department of Gastroenterology and Hepatology, UMass Chan Medical School, Worcester, MA 01655, United States

Ajay Kumar Mishra, Division of Cardiology, Saint Vincent Hospital, Worcester, MA 01608, United States

Corresponding author: Ajay Kumar Mishra, MD, Academic Fellow, Division of Cardiology, Saint Vincent Hospital, 123 Summer Street, Worcester, MA 01608, United States.
ajaybalasore@gmail.com

Abstract

BACKGROUND

The advent of coronavirus disease 2019 (COVID-19) unveiled the worst national blood crisis that the United States had witnessed in over a decade. With the pandemic influencing the different stages of the acquisition of blood products outside the hospital setting, we aimed to explore the possible barriers contributing to the shortage of blood products within the medical community.

AIM

To assess the adherence to restrictive blood transfusion practices for patients in the COVID era and pre-COVID era.

METHODS

We conducted a retrospective cross-sectional study on hospitalized patients distinguishing the pattern of blood transfusion during the COVID and pre-COVID era in a community hospital. Data was tabulated to include the number of red blood cell (RBC) transfusions and if transfusions met restrictive blood transfusion criteria as per institutional guidelines. Chi-square was applied to test

the statistical association between qualitative variables. Unpaired *t* test and Mann Whitney *U* test were applied respectively to test the mean difference of quantitative variables.

RESULTS

A total of 208 patients were included in the study, of which 108 were during COVID era and 100 were during pre-COVID era. The leading reason for admission in both the COVID era and pre-COVID era transfused patients was shortness of breath (53.7% and 36% $P = 0.001$), followed by gastrointestinal bleeding (25.9% and 21% $P = 0.001$). There was a higher percentage of RBC transfusions in the intensive care unit in the COVID-era group than in the pre-COVID era group (38.9% *vs* 22%, $P = 0.008$). The restrictive transfusion criteria were met in 62% *vs* 79% in the COVID and pre-COVID eras, respectively ($P = 0.008$).

CONCLUSION

The COVID-era group received RBC transfusions with less stringent adherence to restrictive blood transfusion practices in comparison to pre-COVID era group.

Key Words: Blood transfusion; Restrictive transfusion; COVID-19; Pre-COVID-19; Blood shortage; Pandemic

Core Tip: Our study showed the percentage of liberal transfusions in a community teaching hospital almost doubled during the coronavirus disease (COVID) era compared to the pre-COVID era, shedding light on a possible change in physician mindset in adhering to restrictive transfusion guidelines during the early COVID-19 pandemic. It reiterates the importance for timely physician education on restrictive red blood cell transfusion guidelines.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) began in the city of Wuhan, China, with the first reported case in December 2019 while the United States recorded the first two cases of the severe acute respiratory syndrome coronavirus 2 in Illinois on January 24, 2020 leading to declaration of national emergency on March 13, 2020. With supply of many commodities across the globe facing challenges, the U.S. Food and Drug Administration released a list of medication shortages in the country which included cardiovascular medications like furosemide, labetalol, and anticoagulants such as heparin[1].

On January 1, 2022, the first day of 'National blood donor month, the American Red Cross, which supplies 40% of the nation's blood, declared the worst national blood crisis in over a decade. The pandemic of COVID-19 impacted the practices of blood product procurement and transfusion in multiple ways[2-5]. The pre-transfusion process was impacted more severely than the clinical transfusion phase[6]. One of the main reasons causing reduction in blood products was social distancing precautions to avoid public gatherings issued during the pandemic by various national governments[7]. A systematic review and meta-analysis showed significantly decreased blood donation rates across the globe during the pandemic[8]. Low donor turnouts limiting the supply of short-lived blood products, compounded the predicament. Resource-limited community medical centers nationwide faced challenges in securing products for blood transfusion[9].

The Association for the Advancement of Blood and Biotherapies (AABB) published the restrictive transfusion guidelines in 2016 (Table 1). The recommendations stemmed from summarizing several trials that demonstrated restrictive red blood cell (RBC) transfusion thresholds to not be harmful compared to the liberal transfusion threshold (transfusing RBCs at higher hemoglobin thresholds- 9 g/dL to 10 g/dL). The restrictive transfusion threshold approach of 7 g/dL or 8 g/dL was synchronous with decreased blood product use and associated cost of hospitalization coupled with no greater impact on rates of adverse clinical outcomes, including 30-day mortality, myocardial infarction, cerebrovascular accident, re-bleeding, pneumonia, or thromboembolism. Though there are definite guidelines for admitted hemodynamically stable patients, the threshold for RBC transfusion is not well defined in a situation of active blood loss or rapidly down trending hemoglobin[10]. This often leads to heterogeneity in guidelines for RBC transfusion in different institutions.

With the pandemic influencing the different stages of the acquisition of blood products outside the hospital setting, we aimed to explore the possible barriers contributing to the shortage of blood products within the medical community. We found a lack of evidence in the literature looking into internal factors contributing to the national blood shortage, specifically exploring the physician component. Hence, we hypothesized that one such factor could be the adherence to restrictive transfusion practice in the COVID era compared to the pre-COVID era. We conducted a retrospective cross-sectional study observing the pattern of RBC transfusions during the COVID and pre-COVID era in a community

Table 1 Restrictive transfusion guidelines from Association for the Advancement of Blood and Biotherapies published in 2016

Transfusion threshold	Patient population
Hemoglobin ≤ 7 g/dL	Hospitalized adult patients who are hemodynamically stable, including critically ill patients
Hemoglobin ≤ 8 g/dL	Patients undergoing orthopedic surgery, cardiac surgery, and those with preexisting cardiovascular disease

These recommendations do not apply to patients with acute coronary syndrome, severe thrombocytopenia (patients treated for hematological or oncological reasons who are at risk of bleeding), and chronic transfusion dependent anemia.

hospital.

MATERIALS AND METHODS

Study design and participants

We performed a retrospective cross-sectional study to compare the adherence to restrictive transfusion practice in patients admitted to at a 329 - bed community teaching hospital in central Massachusetts. The patients who received RBC transfusions from April 1st 2020 to August 31st 2020 were deemed to be in the COVID era and those who received RBC transfusions from July 1st 2019 to November 30th 2019 were in the pre-COVID era, irrespective of their COVID-19 diagnosis. The above duration was chosen to be representative of the time period around the declaration of COVID-19 related national emergency. The study was approved by the institutional review board. The study inclusion criteria consisted of: (1) Age > 18 years; and (2) Received packed RBC transfusion during the hospital course. Patients with hemoglobinopathies, patients undergoing elective or emergent surgeries, active acute coronary syndrome, transfusion-dependent anemias and pregnant patients were excluded. We excluded these patients due to lack of definite RBC transfusion guidelines by AABB. A detailed chart review was conducted for each patient by two different investigators. The data was tabulated to include the number of RBC transfusions and other blood products received in patients admitted to both the medical floors and intensive care unit (ICU). Demographic data included age, gender, ethnicity, admission diagnosis, pre-existing cardiovascular disease (CVD) or prior gastrointestinal bleeding (GIB), use of chronic antiplatelet or anticoagulation such as aspirin, clopidogrel, direct oral anticoagulants (DOACs), vitamin K antagonists, and use of venous thromboembolism (VTE) prophylaxis during hospitalization. The institutional blood transfusion guidelines remained unchanged during the pandemic.

Exposure and outcomes

The documented indication for transfusion was noted, which included: hemoglobin ≤ 7 g/ dL, hemoglobin ≤ 8 g/ dL with pre-existing CVD, hemoglobin ≤ 10 g/dL with active bleeding and hemoglobin ≤ 10 g/dL with down-trending hemoglobin. These documented indications for RBC transfusion were derived from the pre-available options of the transfusion order set for physicians at our institution. We analyzed whether the documented indication met the restrictive blood transfusion criteria as per institutional guidelines (Table 2).

Data gathering and statistical analyses

The data was collected in Microsoft excel and was analyzed using SPSS. The baseline demographic characteristics between the two study populations were assessed using Chi-square to test the statistical association between qualitative variables. Unpaired *t* test and Mann Whitney *U* test were applied respectively to test the mean difference of quantitative variables following normal and non-normal distribution. The level of significance was set at < 5%. The modalities of Medline, PubMed and Embase were utilized to analyze high impact articles relevant to the current field of study and were incorporated in the discussion.

Definitions

(1) COVID era is defined from April 1st 2020 to August 31st 2020; (2) Pre-COVID era is defined from July 1st 2019 to November 30th 2019; (3) Pre-existing CVD is defined as past history of at least one episode of angina irrespective of percutaneous coronary intervention; (4) Active bleed is defined as documented witnessed bleed by nurse/physician or hemodynamic instability attributed to bleeding; and (5) Down-trending hemoglobin is defined as fall in hemoglobin more than or equal to 2 g/dL.

RESULTS

Two hundred fifteen patients were screened, and 208 met the inclusion criteria. There were 108 patients who received RBC transfusions in the COVID era group and 100 patients who received RBC transfusions in the pre-COVID era group. The mean age of patients in the COVID and pre-COVID eras were 68.01 and 70.22 years (P = 0.29), respectively. Gender

Table 2 Restrictive red blood cells transfusion criteria as per institutional guidelines

RBC transfusion threshold	Patient population
Hemoglobin ≤ 7 g/dL	Hemodynamically stable patients on medical floors and ICU
Hemoglobin ≤ 8 g/dL	Hemodynamically stable patients on medical floors and ICU with preexisting CVD[1]
Hemoglobin ≤ 10 g/dL	Active bleed[2]
Hemoglobin ≤ 10 g/dL	Down-trending hemoglobin[3]

[1]Pre-existing cardiovascular disease is defined as past history of at least one episode of angina irrespective of percutaneous coronary intervention.
[2]Active bleed is defined as documented witnessed bleed by nurse/physician or hemodynamic instability attributed to bleeding.
[3]Down-trending hemoglobin is defined as fall in hemoglobin more than or equal to 2 g/dL.
CVD: Cardiovascular disease; ICU: Intensive care unit.

and race were equally distributed between both groups. Pre-existing CVD and prior GIB were equally distributed between both groups (Table 3).

The leading reason for admission in patients who received RBC transfusions in both the COVID era and pre-COVID era was shortness of breath (53.7% and 36% $P = 0.001$), followed by GIB (25.9% and 21% $P = 0.001$) (Figure 1).

In COVID era group, 10 patients were found to have COVID-19 infection confirmed by positive real time polymerase chain reaction. These patients did not qualify for convalescent plasma treatment due to their immunocompetent status. The COVID era group as compared to pre-COVID era group had a lower proportion of patients on chronic anticoagulant or antiplatelet therapy (18.5% and 46% $P = 0.001$). The distribution of patients based on chronic anticoagulant or antiplatelet therapy in COVID and pre-COVID era groups are as follows: aspirin 4.6% *vs* 11%, aspirin and clopidogrel 1.9% *vs* 8%, DOACs 6.5% *vs* 7% and vitamin K antagonists 5.6% *vs* 20% respectively. Similarly, the COVID era group had a lower proportion of patients on pharmacological prophylaxis for VTE compared to the pre-COVID era patients (13.9% and 29% $P = 0.001$). The distribution of patients based on the types of VTE prophylaxis received in COVID and pre-COVID era groups are as follows: unfractionated heparin 6.5% *vs* 22%, low molecular weight heparin 7.4% *vs* 7% and mechanical prophylaxis 84.3% *vs* 63% respectively. There was a higher percentage of RBC transfusions in the ICU in the COVID-19 era group than in the pre-COVID-era group (38.9% *vs* 22%, $P = 0.008$).

Documented indications for RBC transfusions in COVID and pre-COVID era groups were hemoglobin ≤ 7 g/ dL (47.2% *vs* 43%), hemoglobin ≤ 8 g/ dL with pre-existing CVD (12% *vs* 11%), hemoglobin ≤ 10 g/dL with active bleeding (35.2% *vs* 35%) and hemoglobin ≤ 10 g/dL with down-trending hemoglobin (5.6% *vs* 11%) respectively (Figure 2).

Restrictive transfusion criteria were met in 62% of total RBC transfusions in the COVID era *vs* 79% in the pre-COVID era group ($P = 0.008$) (Table 4). The clinical characteristics of patients who did meet the restrictive transfusion criteria in the COVID and pre-COVID eras are noted in Supplementary Table 1.

The majority of patients in both groups received 1-unit PRBC transfusion. The distribution based on the number of units of RBC transfused in COVID-19 and pre-COVID-19 era groups respectively include: one-unit RBC 74.1% *vs* 83%, two units RBC 25.9% *vs* 11%, three units of RBC 0 *vs* 3% and four units of RBC 0 *vs* 3%. Other blood products received during the COVID and pre-COVID eras included platelets 1.9% *vs* 1% and fresh frozen plasma 11.1% *vs* 8%, respectively.

DISCUSSION

Blood transfusions are the most overused in-hospital procedure, and various observational studies show a definite lack of restrictive transfusion practices[11]. Ours is the first retrospective study in a teaching community hospital to evaluate the pattern of blood transfusions during the COVID and pre-COVID era, specifically highlighting the adherence to restrictive blood transfusion practices. Our study showed 21% of total RBC transfusions in the pre-COVID era did not meet our restrictive transfusion guidelines and that the percentage of liberal transfusions almost doubled (37.9%) during the COVID era. Although different hospitals practice restrictive transfusion strategies differently, the transfusion guidelines at our institution were a projection of the restrictive transfusion guidelines from AABB. By comparing two different timelines in the same institution, we aimed to remove biases from heterogeneous institutional practices.

The time period of 4 months for the COVID era was chosen from April 1st 2020 to August 31st 2020 after the declaration of the national emergency in March 2020 and the pre-COVID era from July 1st 2019 to November 30th 2019 was prior to the first case of COVID 19 in December 2019. From prior studies, blood product utilization in a tertiary care hospital did not show statistically significant variation across different time periods in a year[12]. Many hospitals worldwide saw significant reduction in hospitalizations and postponed elective surgeries during the early pandemic which may have contributed to increased blood product inventory[13]. However, our study saw fairly equal number of RBC transfusions in both the COVID ($n = 108$) and pre-COVID era ($n = 100$). The primary presenting clinical complaint in both groups was shortness of breath, with a more significant number in the COVID era group (54% *vs* 36%), due to suspected COVID-19 infection from a sick contact. In our study, fewer patients receiving RBC transfusions in the COVID era group were on chronic antiplatelet/anticoagulant treatment at the time of presentation (18.5% *vs* 46%) and pharmacologic VTE prophylaxis (13.9% *vs* 29%), as compared to the pre-COVID era group. This could be secondary to a lower number of patients with pre-existing CVD and a higher number of patients with prior GIB in the COVID era group compared to the

Table 3 Demographic and clinical characteristics, *n* (%)

Variable	COVID era (*n* = 108)	Pre COVID era (*n* = 100)	*P* value
Demographics			
Mean age, years	68	70	0.295
Sex			
Male	51 (47.2)	49 (49)	0.798
Female	57 (52.8)	51 (51)	0.798
Race[1]			
Caucasian	86 (79.6)	84 (84)	0.758
Hispanics	1 (0.9)	1 (1)	0.758
African American	8 (7.4)	3 (3)	0.758
Asian	2 (1.9)	2 (2)	0.758
Others	11 (10.2)	10 (10)	0.758
Clinical characteristics			
Preexisting CVD	15 (13.9)	29 (29)	0.008
Prior GIB	11 (10.2)	8 (8)	0.585
Active antiplatelet/anticoagulant use	20 (18.5)	46 (46)	0.001
Inpatient VTE prophylaxis[2]	15 (13.9)	29 (29)	0.001
RBC transfusions in ICU	42 (38.9)	22 (22)	0.008

[1]Race was determined by the participants.
[2]Unfractionated or low molecular weight heparin.
CVD: Cardiovascular disease; ICU: Intensive care unit; VTE: Venous thromboembolism; GIB: Gastrointestinal bleeding; COVID: Coronavirus disease.

Table 4 Comparison of number of red blood cells transfusions meeting restrictive transfusion criteria in the coronavirus disease and pre-coronavirus disease era, *n* (%)

Restrictive transfusion criteria met	Number and percentage of red blood cell transfusions	
	COVID era	Pre-COVID era
Yes	67 (62)	79 (79)
No	41 (38)	21 (29)
P value	0.008	

COVID: Coronavirus disease.

pre-COVID era group. There is evidence in the literature to support increased use of anticoagulants during the pandemic, however this does not apply to our study since the study period was prior to the widespread use of therapeutic anticoagulation for admitted COVID-19 positive patients[14,15]. Irrespective of the differences in the patient characteristics between the COVID and the pre-COVID era (Table 3), the transfusion guidelines defined by AABB support adherence to restrictive transfusion criteria.

It is well established that there was a rise in admissions to the ICU during the pandemic and in our study, we observed an increased number of RBC transfusions in the ICU in the COVID era group[16]. Nevertheless, there is no evidence to show if COVID-19 patients had an increased need for blood transfusion; however, studies indicate that critically ill patients with COVID-19 may have required more blood transfusions compared to non-critically ill patients[17]. The Transfusion Requirements in Critical Care (TRICC) trial that the restrictive transfusion strategy is as effective as the liberal transfusion strategy with improved mortality in critically ill patients[18]. The AABB guidelines remain firm on restrictive transfusion practices for hemodynamically stable patients, irrespective of ICU level of care[10].

The adherence to a restrictive transfusion strategy of 7 g/dL to 8 g/dL can help decrease the number of RBC transfusions[19]. Not only does it impact blood product availability logistically, but it also has clinical consequences. Evidence suggests that the liberal transfusion strategy is associated with worsening disease burden by causing circulatory overload and increased thrombogenicity[20,21]. These adverse effects are in addition to the increased risk of hospital-

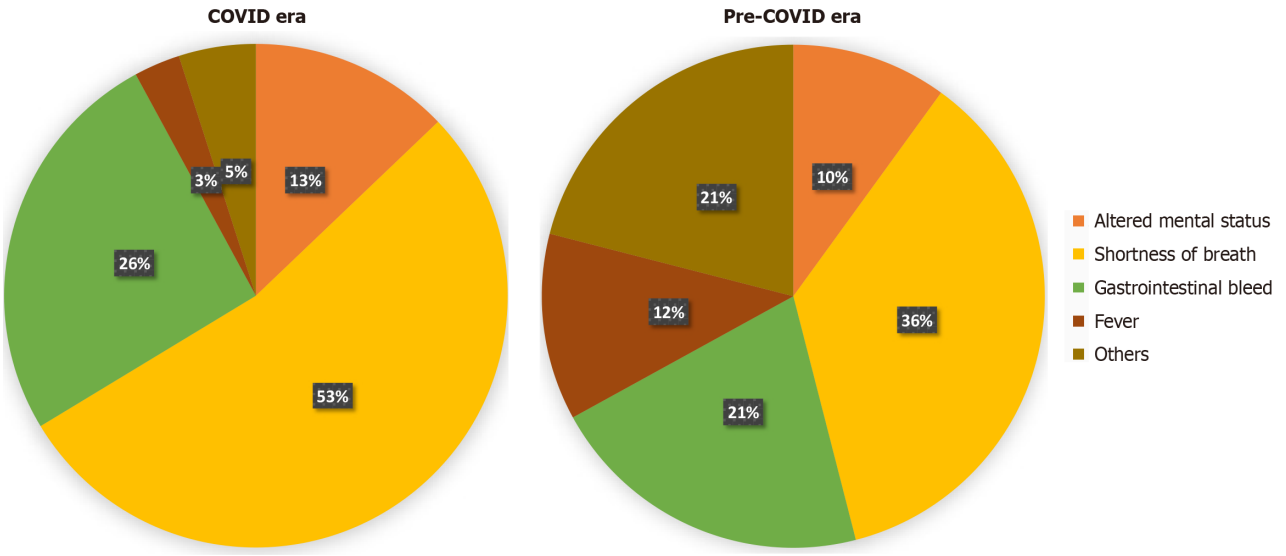

Figure 1 Reason for admission in patients receiving red blood cells transfusion in coronavirus disease and pre-coronavirus disease era. COVID: Coronavirus disease.

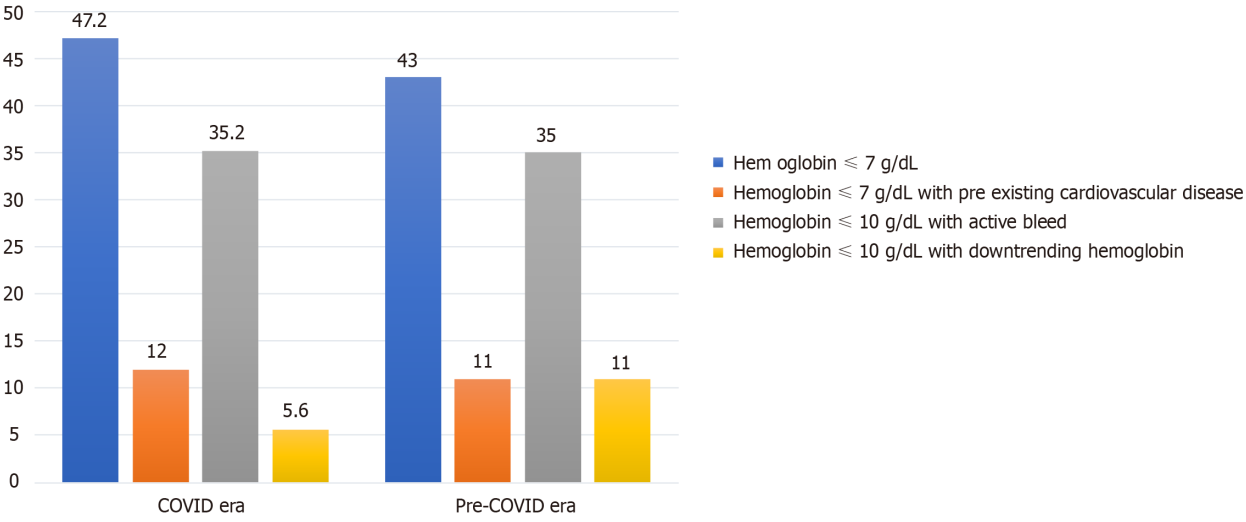

Figure 2 Documented indications for red blood cells transfusion in coronavirus disease and pre-coronavirus disease era. COVID: Coronavirus disease.

acquired infections in liberal transfusion policy as compared to restrictive transfusion policy[22]. The guidelines published by AABB summarize various trials highlighting no greater rate of adverse outcomes in the restrictive transfusion strategy than in the liberal transfusion strategy. Even though RBC transfusions are relatively safe, unnecessary transfusions should be assessed for risk *vs* benefit and associated cost. Given the evidence for the similar safety profile of the restrictive transfusion approach, it necessitates a change in the mindset of the medical community to incorporate the same in clinical practice.

Worldwide, the COVID-19 pandemic brought an era of uncertainty and paranoia. The number of critically ill hospitalized patients and ICU admissions increased dramatically[15,23]. We question whether the pandemic's mortality and paranoia obliged physicians to have a more liberal mindset toward blood transfusions? Did it coerce physicians to practice defensive medicine? As evident from our results, the COVID era group, despite having comparable, if not fewer, risk factors associated with blood loss, received a blood transfusion with less stringent adherence to restrictive blood transfusion guidelines. The results of our study indicate that there could have been more liberal use of blood products while caring for patients during COVID-19 since physicians were still searching for various modalities to approach COVID-19 infection and its related complications[24].

Limitations

Our study was a retrospective cross-sectional study, and given its observational nature, there was a lack of data regarding the clinical judgment made by the physician that preceded the decision to transfuse liberally. Our data is primarily from

one healthcare center.

Future implications

Further studies are needed to pool data from larger geographical locations to comment if liberal transfusion practices during the pandemic was a universal finding. It would also be imperative to see if this trend continued beyond the early pandemic era. Streamlining transfusion practices by being cognizant of the internal factors within the medical community will help prevent a future blood crisis.

CONCLUSION

The role of several external factors that contributed to blood shortages worldwide during the COVID-19 pandemic is well established. However, to our knowledge, ours is the first retrospective study in a tertiary care community teaching hospital to explore the possible role of internal factors within the medical community. Our study sheds light on a possible change in physician mindset in adhering to restrictive transfusion guidelines during the early COVID-19 pandemic. It begs the question of physicians' possible practice of defensive medicine being a preventable cause, though minor, for the National Blood Crisis in 2022. Hence it is pivotal for timely physician education on restrictive RBC transfusion guidelines. There is also a dire need to educate ancillary staff and blood bank associates on these practices, as they would function as critical checkpoints against liberal transfusion policies.

FOOTNOTES

Author contributions: Arun Kumar S, Prabhu S and George SV conceived the idea for the study; Arun Kumar S, Gogtay M, and Singh Y designed and undertook the literature review; Arun Kumar S, Sanghvi A, Suresh MG and Khosla H collected data; Singh Y and Mishra AK performed the statistical analysis, figures, and appendix and analyzed and interpreted the data; Arun Kumar S, Prabhu S, Sanghvi A and Gogtay M wrote the first draft of the manuscript; Arun Kumar S, Suresh MG, Khosla H, Singh Y, Mishra AK and George SV revised the subsequent drafts of the manuscript; all authors reviewed and agreed on the final draft of the manuscript.

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Country of origin: United States

ORCID number: Sumukh Arun Kumar 0000-0001-9689-9978; Sushmita Prabhu 0000-0002-6186-8132; Maya Gogtay 0000-0001-9955-7121; Mithil Gowda Suresh 0000-0002-1495-5444; Harshit Khosla 0000-0003-1192-0825; Yuvaraj Singh 0000-0003-4970-8870; Ajay Kumar Mishra 0000-0003-4862-5053; Susan George 0000-0003-0379-8022.

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ORIGINAL ARTICLE

Retrospective Study

Long-term follow-up of kidney transplant recipients admitted to a tertiary care transplant center with SARS-CoV-2

Emily E Zona, Mina L Gibes, Asha S Jain, Jeannina A Smith, Jacqueline M Garonzik-Wang, Didier A Mandelbrot, Sandesh Parajuli

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Emily E Zona, Mina L Gibes, Asha S Jain, Division of Nephrology, Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI 53705, United States

Jeannina A Smith, Department of Infectious Disease, University of Wisconsin Hospital and Clinics, Madison, WI 53705, United States

Jacqueline M Garonzik-Wang, Department of Surgery, University of Wisconsin, Madison, WI 53705, United States

Didier A Mandelbrot, Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI 53705, United States

Sandesh Parajuli, Department of Medicine, University of Wisconsin School of Medicine and Public Health, University of Wisconsin Madison, Madison, WI 53705, United States

Corresponding author: Sandesh Parajuli, MD, Associate Professor, Staff Physician, Department of Medicine, University of Wisconsin School of Medicine and Public Health, University of Wisconsin Madison, 4175 MFCB1685, Highland Ave, Madison, WI 53705, United States. sparajuli@medicine.wisc.edu

Abstract

BACKGROUND

Kidney transplant recipients (KTR) are at risk of severe coronavirus disease 2019 (COVID-19) disease and mortality after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. We predicted that hospitalization for COVID-19 and subsequent admission to the intensive care unit (ICU) would yield worse outcomes in KTRs.

AIM

To investigate outcomes among KTRs hospitalized at our high-volume transplant center either on the general hospital floor or the ICU.

METHODS

We retrospectively describe all adult KTRs who were hospitalized at our center with their first SARS-CoV-2 infection between 04/2020 and 04/2022 and had at least 12 months follow-up (unless they experienced graft failure or death). The

cohort was stratified by ICU admission. Outcomes of interest included risk factors for ICU admission and mortality, length of stay (LOS), respiratory symptoms at admission, all-cause graft failure at the last follow-up, and death related to COVID-19.

RESULTS

96 KTRs were hospitalized for SARS-COV-2 infection. 21 (22%) required ICU admission. The ICU group had longer hospital LOS (21.8 *vs* 8.6 days, $P < 0.001$) and were more likely to experience graft failure (81% *vs* 31%, $P < 0.001$). Of those admitted to the ICU, 76% had death at last-follow up, and 71% had death related to COVID-19. Risk factors for ICU admission included male sex (aHR: 3.11, 95%CI: 1.04-9.34; $P = 0.04$). Risk factors for all-cause mortality and COVID-19-related mortality included ICU admission and advanced age at SARS-CoV-2 diagnosis. Mortality was highest within a month of COVID-19 diagnosis, with the ICU group having increased risk of all-cause (aHR: 11.2, 95%CI: 5.11-24.5; $P < 0.001$) and COVID-19-related mortality (aHR: 27.2, 95%CI: 8.69-84.9; $P < 0.001$).

CONCLUSION

ICU admission conferred an increased risk of mortality, graft failure, and longer LOS. One-fifth of those hospitalized died of COVID-19, reflecting the impact of COVID-19-related morbidity and mortality among KTRs.

Key Words: Kidney transplants; SARS-CoV-2; Intensive care unit admission; Outcomes; Graft failure

Core Tip: This retrospective study investigated risk factors and outcomes among kidney-only transplant recipients who were diagnosed with and hospitalized for severe acute respiratory syndrome coronavirus 2 infection at a large volume transplant center within the first two years of the coronavirus disease 2019 (COVID-19) pandemic. Recipients were divided into two groups based on whether they were admitted and/or transferred to the intensive care unit (ICU) or the general care floors. Recipients admitted to the ICU had longer hospital length of stays, higher risk of graft failure, and higher all-cause and COVID-19-related mortality rates compared to the general care group. Male sex was a risk factor for ICU admission.

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INTRODUCTION

According to the World Health Organization, as of first week of February 2024, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) resulted in 774593066 confirmed cases and claimed the lives of 7028881 individuals worldwide[1]. SARS-CoV-2 has had a disproportionate and devastating impact on immunocompromised populations who are at increased risk of severe coronavirus disease 2019 (COVID-19) disease and adverse outcomes[2]. Early studies among solid organ transplant recipients (SOTRs) reported a mortality rate of approximately 20%, which was higher than the general population[3]. Some studies even report (KTRs) have the highest mortality among SOTRs[4].

Although the current literature has identified many risk factors associated with negative outcomes among KTRs with COVID-19, few have studied this among those hospitalized with the disease. A single-center cohort study of 2500 KTRs found that vaccinated patients had improved outcomes concerning mortality, hospitalization, and intensive care unit (ICU) admission[5]. In another single-center study of 400 KTRs that compared unvaccinated and vaccinated recipients, there were no significant differences in the rates of ICU admission, length of hospital stay, death, or graft failure, although the incidence of SARS-CoV-2 infection was higher in the unvaccinated group[6]. Another study found that the severity of COVID-19 disease necessitating the hospitalization of KTRs was predominantly influenced by comorbid conditions and baseline kidney function [baseline estimated glomerular filtration rate (eGFR)]; however, they found that when adjusted for comorbidity and renal function, they found no statistically significant differences in mortality, ICU admission, and length of stay between adults hospitalized for COVID-19 who had undergone kidney transplant and non-transplanted counterparts[7]. Further study is necessary to understand risk factors and outcomes for ICU admission in this vulnerable population.

To explore this further, we sought to investigate outcomes among KTRs hospitalized at our high-volume transplant center either on the general hospital floor or the ICU. Outcomes of interest were risk factors for ICU admission, risk for all-cause mortality and COVID-19-related mortality.

MATERIALS AND METHODS

Patients

We evaluated all adult (> 18 years of age) kidney-only transplant recipients at the University of Wisconsin who were subsequently diagnosed with and hospitalized for SARS-CoV-2 infection between April 2020 and April 2022. We excluded individuals who were followed at our center but did not have a functioning allograft. Given that in April 2020 the first case of the SARS-CoV-2 was isolated in our kidney transplant recipient population, April 2020 was selected as the start date for this review. All recipients had at least one year of follow-up unless they had allograft failure or death. Multiorgan transplant recipients or recipients less than 18 years of age at the time of diagnosis of SARS-CoV-2 infection were excluded.

Recipients were divided into two groups based on whether they were admitted/transferred to the ICU or not. Risk factors for ICU admission, all-cause mortality, and COVID-19-related mortality were outcomes of interest. Additionally, we evaluated for changes in allograft function with serum creatinine and eGFR at various periods post-SARS-CoV-2 infection. During hospitalization, monitoring of other non-invasive biomarkers was not routinely performed.

This study was approved by the University of Wisconsin School of Medicine and Public Health Institutional Review Board (IRB protocol number: 2014-1072). This study was in adherence to the Declaration of Helsinki. The clinical and research activities being reported were consistent with the Principles of the Declaration of Istanbul as outlined in 'The Declaration of Istanbul on Organ Trafficking and Transplant Tourism'. Due to the nature of the study (retrospective, observational) informed consent pertinent to this study was not obtained from the recipients.

We follow our kidney transplant recipients at either the University Hospital or various regional outreach clinics at least once a year until graft failure or until the patient decides to transfer their care to a different center as previously described. All major health events were documented in our master database and were included in the study.

Definition of variables

COVID-19-related death was defined as SARS-CoV-2 infection as the cause of mortality as documented in the electronic health record. Every death occurred in the setting of a functional graft. Death-censored graft failure was characterized by either the necessity to resume dialysis or undergo re-transplantation. All rejections were confirmed through biopsy. Vaccination was defined as receiving at least one dose of an available SARS-CoV-2 vaccine. ICU admission was defined as any admission or transfer to the ICU for greater than or equal to 1 day. Indications for ICU admission for SARS-CoV-2 paralleled with any other indications for ICU admission including severe respiratory distress needing high flow oxygen or intubation, hemodynamical instability, and many more.

Immunosuppression

As previously described, the majority of KTRs are managed with a triple immunosuppressant regimen, primarily comprising tacrolimus, mycophenolic acid, and prednisone[8,9]. A minority of KTRs underwent early steroid withdrawal or received alternative immunosuppression regimens. Once the patient is admitted to the ICU, all regular maintenance immunosuppressive are usually held and recipients are maintained on high-dose intravenous steroids.

SARs-CoV-2 infection: Clinical management

Our KTRs are urged to inform the transplant center upon testing positive for or being suspected of having COVID-19. If they test positive, we advise them to pursue early treatment for the disease. Furthermore, patients are encouraged to seek urgent evaluation if their oxygen saturation falls below 90%, or if they encounter worsening shortness of breath, inability to hydrate due to vomiting or diarrhea, or altered mental status.

Statistical analyses

Categorical data were analyzed using Fisher's exact test or chi-square test, while continuous data were compared with the Student's *t*-test or the Wilcoxon rank-sum test. *P* values ≤ 0.05 were regarded as statistically significant. Risk factors linked to ICU admission, all-cause mortality, and COVID-19-related mortality were examined through univariate and multivariate stepwise Cox regression analyses. Variables showing associations with outcomes at a significance level of $P \leq 0.10$ in the univariate analysis were retained for inclusion in the multivariate analyses. Kaplan-Meier analyses were used to analyze all-cause mortality and COVID-19-related mortality.

RESULTS

96 KTRs received a SARS-CoV-2 infection diagnosis. Of these, 21 (22%) needed ICU admission and 75 (78%) received general hospital-based care. All KTRs included in this study were admitted to our University hospital. The median interval from COVID-19 to the last follow-up in those requiring ICU admission was 0.71 (IQR: 0.49-1.10) months and those who did not requiring ICU admission was 17.04 (IQR: 12.6-28.3) months ($P < 0.001$). Table 1 summarizes the baseline characteristics. Baseline serum creatinine before COVID-19 diagnosis was higher in the non-ICU group ($P < 0.001$), but otherwise there were no differences between the two groups.

Table 2 compares various outcomes between recipients admitted to the ICU and those admitted to general hospital care. Mean hospital LOS was greater in the ICU group (21.8 ± 19.4) compared with the non-ICU group (8.6 ± 9.8). At last follow-up, patients admitted to the ICU were more likely to experience graft failure and death compared with the non-

Table 1 Baseline characteristics, *n* (%)

Characteristics		ICU admission, *n* = 21	Non-ICU admission, *n* = 75	*P* value
Age at transplant (years)		54.7 ± 13.9	50.4 ± 15.2	0.68
Age at COVID-19 diagnosis (years)		59.5 ± 14.1	57.1 ± 14.4	0.97
Male		16 (76)	40 (53)	0.06
Nonwhite		4 (19)	20 (27)	0.50
Cause of ESKD	Diabetes mellitus	6 (28)	21 (28)	0.56
	Hypertension	4 (19)	9 (12)	
	Glomerular disease	8 (38)	23 (31)	
	Polycystic kidney disease	0	6 (8)	
	Other	3 (14)	16 (21)	
Living donor		3 (14)	28 (37)	0.04
Previous transplant		3 (14)	23 (31)	0.14
Maintenance immunosuppressive	Tacrolimus + Mycophenolic acid + prednisone	18 (86)	53 (71)	0.17
	Prednisone based immunosuppression	19 (91)	69 (91)	0.98
Vaccinated		11 (52)	44 (59)	0.61
Rejection within six months before COVID-19		0	2 (3)	0.45
Baseline serum creatinine before COVID-19 (mg/dL)		1.57 ± 0.73	1.89 ± 1.57	< 0.001
Baseline eGFR before COVID-19 (mL/m^2)		53.7 ± 21.1	42.3 ± 19.5	0.61
The interval from transplant to the first COVID-19 (month)		58.5 ± 54.7	81.8 ± 68.4	0.26

COVID-19: Coronavirus disease 2019; eGFR: Estimated glomerular filtration rate; ICU: Intensive care unit; ESKD: End-stage kidney disease.

ICU admission group (P < 0.001). 17 recipients (81%) in the ICU group experienced graft failure compared with 23 recipients (31%) in the non-ICU group. Notably, of the 16 recipients (76%) in the ICU group who had death at last follow-up, 15 (71%) of these deaths were because of COVID-19. In the non-ICU group, 4 (5%) of the 13 (17%) deaths at last follow-up were primarily attributed to COVID-19.

Assessing the risk factors for ICU admission (Table 3), male sex was the only factor significantly associated with increased risk for ICU admission in the univariate (HR: 3.29; 95%CI: 1.10-9.88; P = 0.03) and multivariate (HR: 3.29; 95%CI: 1.10-9.88; P = 0.04) analyses. Interestingly, vaccination (HR: 0.93; 95%CI: 0.38-2.25; P = 0.87) and living donor recipient (HR: 0.35; 95%CI: 0.10-1.19; P = 0.09) were equally represented in patients admitted to the ICU and to the floor.

Furthermore, examining the risk of all-cause mortality (Table 4) in univariate analysis, ICU admission (HR: 10.2; 95%CI: 4.72-21.9; P < 0.001) and advanced age (HR: 1.05; 95%CI: 1.02-1.08; P = 0.002) were associated with increased risk. This finding carried over into the multivariate analyses as well. ICU admission was associated with an 11-fold increase in the risk of all-cause mortality, especially evident during the early post-SARS-CoV-2 infection period, as illustrated in Figure 1A.

COVID-19-related mortality risk showed similar outcomes (Table 5 and Figure 1B). In the univariate analysis, ICU admission (HR: 25.5; 95%CI: 8.26-78.8; P < 0.001) was associated with increased risk for COVID-19-related mortality, but this time by over 25-fold. This increased risk associated with ICU admission persisted in the multivariate analysis as well (HR: 27.2; 95%CI: 8.69-84.9; P < 0.001). Though advanced age was not quite statistically significant in the univariate analysis (P = 0.06), it was significantly associated with increased risk in the multivariate analysis (HR: 1.04; 95%CI: 1.00-1.08; P = 0.04). Vaccination, male sex, nonwhite recipients, diabetes as the cause of end-stage kidney disease (ESKD), living donor recipients, and baseline eGFR conferred neither increased nor decreased risk for COVID-19-related mortality once hospitalized with COVID-19.

DISCUSSION

Among this group of 96 KTRs admitted to a single institution during the initial two years of the COVID-19 pandemic, we presented important features associated with SARS-CoV-2 infection and its outcomes. This study distinguishes hospitalization based on whether the KTRs required ICU admission, allowing us to further characterize risk factors and outcomes according to this variable. To summarize, KTRs admitted to the ICU had a higher mean hospital LOS, higher risk of graft failure, and higher mortality rate compared to the non-ICU admission group. 81% of KTRs in the ICU group experienced graft failure, a stark contrast to the 31% rate of graft failure in the non-ICU group. Additionally, 15 out of 16 KTRs (94%)

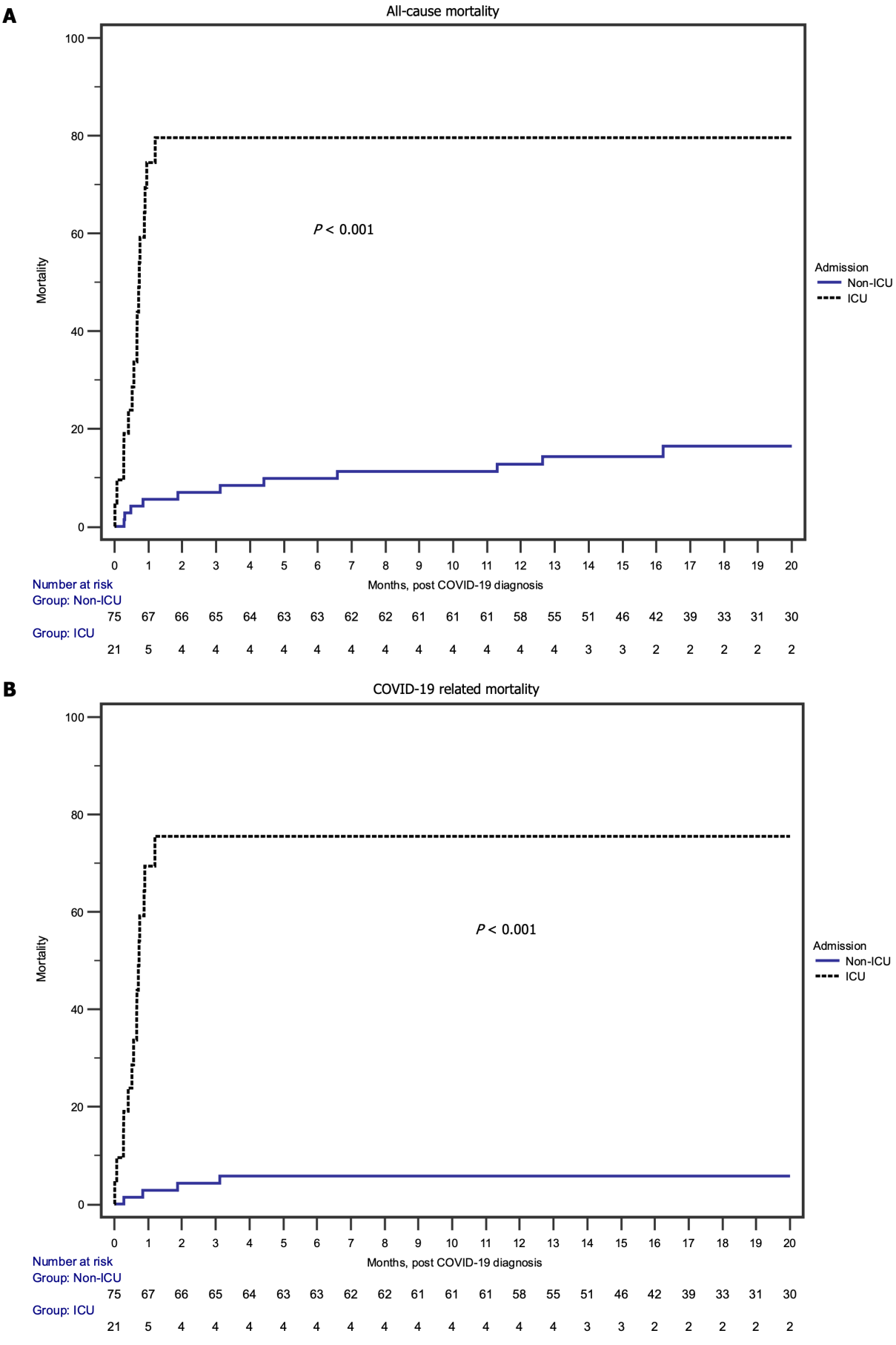

Figure 1 Mortality. A: All-cause mortality; B: Coronavirus disease 2019-related mortality. ICU: Intensive care unit; COVID-19: Coronavirus disease 2019.

Table 2 Outcomes, *n* (%)

Characteristics	ICU admission	Non-ICU admission	*P* value
Respiratory symptoms for admission	15 (71)	49 (65)	0.6
Mean hospital length of stay (days)	21.8 ± 19.4	8.6 ± 9.8	< 0.001
Use of remdesivir for management of SARS-CoV-2	5 (24)	23 (31)	0.54
Serum creatinine at time of SARS-CoV-2 infection (mg/dL)	1.91 ± 0.25	2.50 ± 2.17	0.002
Serum eGFR at the time of SARS-CoV-2 infection (mL/m^2)	48.1 ± 24.6	37.9 ± 21.1	0.34
Serum creatinine 1-month post SARS-CoV-2 infection (mg/dL)	1.22 ± 0.29 (*n* = 6)	1.86 ± 1.47 (*n* = 66)	0.12
Serum eGFR 1 month SARS-CoV-2 infection (mL/m^2)	73 ± 30.7	46.4 ± 21.7	0.18
Serum creatinine 6 months post SARS-CoV-2 infection (mg/dL)	1.34 ± 0.62 (*n* = 5)	1.67 ± 0.72 (*n* = 62)	0.86
Serum eGFR 6 months SARS-CoV-2 infection (mL/m^2)	67.0 ± 32.8	47.0 ± 19.5	0.07
Serum creatinine 1 year post SARS-CoV-2 infection (mg/dL)	1.32 ± 0.70 (*n* = 4)	1.93 ± 1.21 (*n* = 61)	0.39
Serum eGFR 1 year post SARS-CoV-2 infection (mL/m^2)	70.3 ± 34.9	44.3 ± 20.8	0.09
Serum creatinine at last follow-up (mg/dL)	1.29 ± 0.84 (*n* = 4)	1.88 ± 1.30 (*n* = 53)	0.5
Serum eGFR at last follow-up (mL/m^2)	76.8 ± 37.0	48.1 ± 21.9	0.1
Uncensored graft failure at last follow-up	17 (81)	23 (31)	< 0.001
Death at last follow-up	16 (76)	13 (17)	< 0.001
Death related to COVID-19	15 (71)	4 (5)	< 0.001

COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; eGFR: Estimated glomerular filtration rate; ICU: Intensive care unit.

Table 3 Risk factors for intensive care unit admission

Covariate	Univariate analyses			Multivariate analyses		
	HR	95%CI	*P* value	HR	95%CI	*P* value
Age at SARS-CoV-2 infection diagnosis (per year)	1.01	0.98-1.05	0.35			
Male recipient	3.29	1.10-9.88	0.03	3.11	1.04-9.34	0.04
Nonwhite recipient	0.71	0.24-2.13	0.55			
Diabetes as a cause of ESKD *vs* other	1.09	0.42-2.83	0.86			
Living donor recipient	0.35	0.10-1.19	*0.09*	0.38	0.11-1.30	0.12
Previous transplant	0.46	0.13-1.55	0.2			
Tacrolimus + MPA + prednisone maintenance *vs* other	2.07	0.61-7.08	0.24			
Prednisone based immunosuppression	0.89	0.21-3.82	0.87			
Treatment of rejection before SARS-CoV-2 infection	--	--	--			
Vaccinated	0.93	0.38-2.25	0.87			
Baseline eGFR pre- SARS-CoV-2 infection (per mL/m^2)	1.01	0.99-1.03	0.23			
The interval from transplant to COVID-19 (per month)	0.99	0.98-1.01	0.4			

COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; eGFR: Estimated glomerular filtration rate; ESKD: End-stage kidney disease; MPA: Mycophenolic acid.

Table 4 Risk for all-cause mortality

Covariate	Univariate analyses			Multivariate analyses		
	HR	95%CI	*P* value	HR	95%CI	*P* value
ICU admission	10.2	4.72-21.9	< 0.001	11.2	5.11-24.5	< 0.001
Age at COVID-19 diagnosis (per year)	1.05	1.02-1.08	0.002	1.06	1.02-1.09	0.001
Male recipient	1.90	0.86-4.17	0.11			
Nonwhite recipient	0.60	0.23-1.58	0.31			
Diabetes as a cause of ESKD *vs* other	1.13	0.52-2.50	0.75			
Living donor recipient	0.72	0.32-1.64	0.44			
Previous transplant	0.66	0.27-1.61	0.36			
Tacrolimus + MPA + prednisone maintenance *vs* other	1.18	0.51-2.78	0.69			
Prednisone based immunosuppression	0.87	0.26-2.87	0.82			
Treatment of rejection before SARS-CoV-2 infection	--	--	--			
Vaccinated	0.81	0.39-1.69	0.58			
Baseline eGFR (per mL/m^2)	1.0	0.98-1.02	0.94			
Interval from transplant to COVID-19 (per month)	1.0	0.99-1.01	0.73			
Respiratory symptoms for hospital admission	1.22	0.55-2.68	0.63			
Remdesivir for management of COVID	0.48	0.18-1.26	0.14			

COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; eGFR: Estimated glomerular filtration rate; ICU: Intensive care unit; ESKD: End-stage kidney disease; MPA: Mycophenolic acid.

Table 5 Risk for coronavirus disease 2019-related mortality

Covariate	Univariate analyses			Multivariate analyses		
	HR	95%CI	*P* value	HR	95%CI	*P* value
ICU admission	25.5	8.26-78.8	< 0.001	27.2	8.69-84.9	< 0.001
Age at SARS-CoV-2 infection diagnosis (per year)	1.04	0.99-1.07	*0.06*	1.04	1.0-1.08	0.04
Male recipient	1.75	0.66-4.59	0.26			
Nonwhite recipient	0.54	0.16-1.85	0.33			
Diabetes as a cause of ESKD *vs* other	0.66	0.22-1.99	0.46			
Living donor recipient	0.68	0.25-1.90	0.47			
Previous transplant	0.48	0.14-1.66	0.25			
Tacrolimus + MPA + prednisone maintenance *vs* other	1.33	0.44-4.02	0.61			
Prednisone based immunosuppression	0.52	0.15-1.77	0.30			
Treatment of rejection before SARS-CoV-2 infection	--	--	--			
Vaccinated	0.66	0.26-1.60	0.35			
Baseline eGFR (per mL/m^2)	1.01	0.99-1.03	0.27			
Interval from transplant to SARS-CoV-2 infection (per month)	1.0	0.99-1.01	0.68			
Respiratory symptoms for hospital admission	1.13	0.43-2.99	0.80			
Remdesivir for management of COVID	0.62	0.21-1.88	0.40			

COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; eGFR: Estimated glomerular filtration rate; ICU: Intensive care unit; ESKD: End-stage kidney disease; MPA: Mycophenolic acid.

in the ICU group who had death at the last follow-up experienced death attributed to COVID-19. Again, this contrasts with the 4 out of 13 KTRs (31%) in the non-ICU group who had death at the last follow-up experienced death attributed to COVID-19. ICU admission increased risk of all-cause mortality by 11-fold and increased risk of COVID-19-related mortality by 27-fold. Surprisingly, vaccination, living donor recipients, diabetes as the cause of ESKD, and baseline eGFR were neither associated with increased nor decreased risk for ICU admission, all-cause mortality, and COVID-19-related mortality. Unsurprisingly, advanced age significantly increased risk for ICU admission, all-cause mortality, and COVID-19-related mortality in this study.

Advanced age has been widely documented in the literature as an important risk factor for severe COVID-19 disease and mortality[5,9]. While our study is consistent regarding the variable of advanced age, these data diverge from other studies regarding the impacts of vaccination status, comorbid conditions, and immunosuppressive regimens. Many studies document the protective effect of vaccination in reducing the risk of morbidity and mortality from COVID-19 disease in KTRs. One study of 2500 KTRs found that the number of SARS-CoV-2 vaccination doses was inversely correlated with mortality, hospitalization, ICU admission, and acute kidney injury (AKI)[5]. On the other hand, a single-center study of 400 KTRs found no statistically significant differences between unvaccinated and vaccinated KTRs in terms of hospitalization (outpatient, general floor, ICU), length of stay, death, and graft failure[6]. It is important to note that in the study which noted a significant influence in vaccination on outcomes that it analyzed results according to the different SARS-CoV-2 variants, namely pre and post Omicron. This same study also showed that a steroid-free immunosuppressive regimen was a protective factor against hospitalization for COVID-19 disease, COVID-19-related AKI, and ICU admission, with other immunosuppressive agents not being significantly associated with CVOID-19 mortality or morbidity[5]. In contrast to this finding, our study showed no difference in ICU admission among KTRs who received prednisone-based immunosuppression. Regarding graft function, we found that KTRs admitted to the ICU had an increased risk of graft failure. This finding is consistent with results from a study that revealed a sex-specific association between relative eGFR decline and COVID-19 severity among male KTRs.

Current guidelines for the treatment of solid organ transplant recipients with severe and critical COVID-19 recommend intravenous remdesivir with or without immunomodulation agents including dexamethasone, tocilizumab, or baricitinib [10]. Our findings in this study did not show a mortality benefit associated with remdesivir for management of COVID-19. One study of SOTRs hospitalized with SARS-CoV2 infection found that severe COVID-19 cases show high mortality despite antiviral treatment with RDV[11]. Another cohort study found that remdesivir protected KTRs from severe COVID-19 disease only in those hospitalized with early administration of the drug (within 7 days of symptom onset)[12].

As a single-center observational study, this research carries the limitations associated with such a design which include the specific clinical approach and population of our institution. This consistency of the clinical practices reinforces the strength of the study, as all participants were hospitalized at the same center, reducing the risk of confounding variables associated with hospitalization at different locations. As our center is a tertiary care university academic institution, patients admitted or transferred to our center are usually very sick.

CONCLUSION

In conclusion, our patients, especially SOTRs, continue to suffer from the adverse effects of SARS-CoV-2 infection. We remain motivated to continue rigorous efforts to better understand and characterize risk factors and protective factors related to COVID-19 such that we can prevent devastating outcomes and promote the health and safety of our patients.

FOOTNOTES

Author contributions: Zona EE was responsible for data collection, manuscript preparation, and editing; Gibes ML and Jain AS were responsible for data collection and editing; Smith JA, Garonzik-Wang J, and Mandelbrot DA were responsible for editing; Parajuli S was responsible for data collection, concept design, analysis, and manuscript preparation; All authors have read and approve the final manuscript.

Institutional review board statement: Tis study was approved by the University of Wisconsin School of Medicine and Public Health Institutional Review Board (IRB protocol number: 2014-1072-CR004). Tis study was in adherence to the Declaration of Helsinki. The clinical and research activities being reported were consistent with the Principles of the Declaration of Istanbul as outlined in "The Declaration of Istanbul on Organ Trafficking and Transplant Tourism."

Informed consent statement: Due to the nature of the study, informed consent from the subject pertinent to this study was not obtained from the subject.

Conflict-of-interest statement: All authors declare no conflicts of interest.

Data sharing statement: The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Country of origin: United States

ORCID number: Didier A Mandelbrot 0000-0003-3326-8583; Sandesh Parajuli 0000-0003-1667-7465.

Corresponding Author's Membership in Professional Societies: American Society of Transplantation; American Society of Nephrology.

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ORIGINAL ARTICLE

Observational Study

Parents's knowledge and awareness about hepatitis B can influence the vaccination of their children

Nanda Chhavi, Geetika Srivastava, Mariya Waseem, Abhishek Yadav, Surender Singh, Rajani Singh, Amit Goel

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Nanda Chhavi, Geetika Srivastava, Mariya Waseem, Department of Paediatrics, Era's Lucknow Medical College, Lucknow 226003, Uttar Pradesh, India

Abhishek Yadav, Medical Consultant, National Disease Control Program, National Health Mission, Lucknow 226003, Uttar Pradesh, India

Surender Singh, Rajani Singh, Amit Goel, Department of Hepatology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, Uttar Pradesh, India

Corresponding author: Amit Goel, BSc, DNB, MBBS, MD, MNAMS, Professor, Department of Hepatology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Raebareli Road, Lucknow 226014, Uttar Pradesh, India. agoel.ag@gmail.com

Abstract

BACKGROUND

Birth-dose (Hep-BD) followed by three additional doses (Hep-B3) of hepatitis B virus (HBV) vaccine are key to eliminating HBV by 2030. Unfortunately, Hep-BD and Hep-B3 coverage in our country is poor.

AIM

To studied the parent's knowledge and awareness about HBV infection, its prevention, consequences and vaccination.

METHODS

Parents of 6 months to 8 years old children were interviewed to assess their knowledge & awareness about hepatitis B, its transmission, prevention, illness caused by this, and vaccination. Eighteen close-ended questions were administered, and responses were recorded as 'yes', 'no', or 'not sure'. HBV knowledge score was calculated based on the sum of correct answers. Each correct response scored one point and incorrect, missing or 'not sure' responses received no points. Categorical data are presented as number (%) and numerical data are expressed as median. Data were compared using Chi^2 tests and level of significance was kept as $P < 0.05$.

RESULTS

Parents (58.3% mothers) of 384 children (89.9% age < 5 years; 82% age-appropriately vaccinated) were included. Three hundred and twenty-two (83.9%)

children were Hep-B3 vaccinated. 94.3%, 87.5%, and 29.2% parents knew about polio, tetanus, and hepatitis B vaccine. Overall, 41.2%, 15.8%, and 23% parents knew about hepatitis B transmission, consequences of infection, and prevention respectively. Only 7.6% parents knew about three-dose schedule of hepatitis B vaccination. Only 23% parents believed that vaccine could prevent HBV, 15.7% knew that HBV affects liver. Parents of Hep-B3 vaccinated children were significantly more aware about HBV than the parents of unvaccinated children ($P < 0.05$ for 17/18 questions).

CONCLUSION

The knowledge and awareness among the parents about hepatitis B is poor. The Increasing knowledge/awareness about HBV among parents may improve Hep-B3 vaccination coverage.

Key Words: Hepatitis B; Viral hepatitis; Cirrhosis; Hepatocellular carcinoma; Hepatotropic viruses; Transfusion transmitted infection; Mother to child transmission

Core Tip: Hepatitis B infection can be prevented with vaccination. Birth dose followed by three doses of hepatitis B vaccine in infancy is one of the key intervention to prevent hepatitis B transmission. Unfortunately, the coverage of hepatitis B vaccination among newborns are not adequate in India. Our article identified that the parents have poor knowledge about the hepatitis B and increasing knowledge/awareness about hepatitis B virus among parents may improve Hepatitis B vaccine coverage in the country.

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INTRODUCTION

Hepatitis B virus (HBV) infection is common in India. Chronic hepatitis B (CHB) infection could lead to liver cirrhosis and hepatocellular carcinoma. Globally, over two billion people have evidence of past HBV infection, 360 million are living with CHB, and 600000 people die annually from the consequences of HBV infection[1]. Recent estimates suggest that 2.9% people in India have HBV infection[2] and it accounts for approximately 11% of cirrhosis burden in the country[3].

HBV infection progresses to CHB in approximately 90% of infants, approximately 20% in children of 1–5 years of age, and 5%–10% in children over 5 years of age and adults[4]. Globally, most of the HBV infections to children are transmitted from their mothers (MTCT), either in-utero or in perinatal period. The MTCT of HBV can largely be prevented with hepatitis B vaccine given at birth (Hep-BD) followed by three doses (Hep-B3) of primary vaccination schedule. This is commonly given as a part of childhood immunization.

The World Health Organization (WHO) aims to eliminate HBV as a public health problem by the end of year 2030. Hepatitis B elimination is defined as 90% and 65% reduction in new HBV cases and HBV related deaths respectively from baseline estimates of 2015. Successful elimination of HBV can be achieved by attaining the proposed WHO targets. One of the targets is to vaccinate 90% or more newborns with Hep-BD and Hep-B3[5].

Though we are only a few years away from 2030, the Hep-BD and Hep-B3 coverages in our country is far less than the proposed target of ≥ 90%. Several factors are responsible for the inadequate HBV vaccination in our country. Inadequate knowledge and awareness of the parents about the HBV may be one of them[6]. Data are limited to the awareness and knowledge of the parents about HBV. Our aim was to study the association of parents' knowledge and awareness about HBV infection, its prevention, and sequelae with the hepatitis B vaccination status of their children.

MATERIALS AND METHODS

Study design and participants

Prospective, single arm, observational, cross-sectional study was conducted in Era's Lucknow Medical College and Hospital, Lucknow, India. Participants were enrolled between March 2020-December 2021.

Parents accompanying their 6 months to 8 years old children in out-patient clinic were screened and eligible parents were included after written informed consent for participation and publication of data. Parents with one or more HBsAg positive family members on either of the parent's side were excluded. If both the parents were available for participation, then data were collected from only mother.

Data collection

Data were collected in a predefined data collection form which had three sections; first section explored the awareness of the parents about the various vaccine given to prevent common childhood illness; second section included data from the vaccination card of the child to assess the vaccination status of the child; third section, explored the parents' knowledge and awareness about HBV infection, its prevention and transmission. The data collection form was completed by the parents with the help of a physician in an isolated and silent place and sufficient time was given to understand and respond.

We applied close-ended questions and responses were recorded as 'yes', 'no', or 'not sure'. It took 10-15 min to complete the data collection form. The knowledge and awareness of the parents on HBV were assessed about disease epidemiology (three questions), routes of transmission (seven question), consequences of its infection (four questions), prevention (three questions), and treatment (one question). The questions were drafted after discussion with faculty members, and the viral hepatitis experts, who were working at various level of seniority. Before starting the study, we piloted our questionnaire in 25 parents and made appropriate changes.

Statistical analysis and ethical considerations

HBV knowledge score of a participant was calculated based on the sum of correct answers given to the set of 18 questions. A correct response to each question received one point. Responses recorded as either incorrect, not sure, or missing scored zero point. Data in various domains were summarized as median. Categorical data are presented as number (%) and numerical data are expressed as median (interquartile range). Data are compared using χ^2 tests and Mann-Whitney *U* tests with level of significance kept as $P < 0.05$.

Study was approved by the institute ethic committee (ELMC & H/R_Cell/EC/2020/20).

RESULTS

Participant characteristics

Of the 408 parents who participated in the study, 24 were excluded because of incomplete data ($n = 16$) or having HBsAg positive family member ($n = 8$). Data from the remaining 384 were analyzed. Demographic characteristics of the parents and their children are summarized in Table 1. In our cohort, 58.3% respondents were mothers, and 89.9% children were of < 5 years of age. Over 86% children had received their primary vaccination primarily in government hospital. Among children, 315 (82%) were age appropriately vaccinated at the time of interview of their parents, and 322 (83.9%) had received all the three doses of Hep-B3.

Knowledge assessment of participants about vaccines in general and hepatitis B vaccine

Overall, 93.5% parents were aware that vaccines are given to prevent infections. Though a large proportion of parents were aware about vaccines against polio (94.3%) and tetanus (87.5%), only 29.2% parents had awareness about hepatitis B vaccine. Among Hep-B3 vaccinated children, 29.5% parents were aware about hepatitis B vaccine.

Parents were asked about the illness for which vaccines are given during the childhood. Their awareness was assessed by asking whether a vaccine is given for a particular childhood illness or not? The responses of the parents are summarized in Table 2. The awareness was highest for polio (94.3%) vaccine followed by tetanus (87.5%) and diarrhea (70%). Only 29.2% parents were aware that hepatitis B vaccine is also included in childhood immunization schedule.

Of the 384 participants, 41.2% were aware about the routes of transmission, 15.8% had knowledge about the consequences of HBV infection, and 23% knew about prevention. Only 7.6% parents knew that three doses of HBV vaccine are given (Table 3). However, only 23% parents believed that vaccine could effectively prevent HBV infection. Further, only 15.7% of parents knew that HBV affects liver (Figure 1). None of the parents could correctly answer all eighteen questions.

Demographic characteristics of the children with or without Hep-B3 vaccination were comparable (Table 4). As compared to the children without Hep-B3 vaccination, a significantly higher proportion of Hep-B3 vaccinated children were delivered in hospital (87% *vs* 96%; $P < 0.01$) and had received vaccines in a private clinic (73% *vs* 89%; $P < 0.01$). The parents of Hep-B3 vaccinated children were significantly more aware about hepatitis B than parents of Hep-B3 unvaccinated children (Table 5).

DISCUSSION

The study showed that though 84% of children were vaccinated with Hep-B3 only 29% parents were aware about hepatitis B vaccine, and 7.6% knew about Hep-B3 vaccination schedule. This shows that the parents had poor faith on effectiveness of hepatitis B vaccine and only 23% believed that vaccine can prevent HBV infection. Overall, the knowledge about hepatitis B was poor among parents as none could correctly answer all the questions and only 15.7% knew that HBV affects liver.

The Expanded Program of Immunization (EPI) started in 1978, laid the foundation of childhood vaccination program in India. EPI provide vaccination for six vaccine preventable diseases, namely diphtheria, pertussis, tetanus, polio, tuberculosis, and measles. The Government of India accepted the WHO recommendation and included Hep-B3 in EIP in 2002 and gradually expanded to the entire country under the aegis of Universal Immunization Program. As of now, our

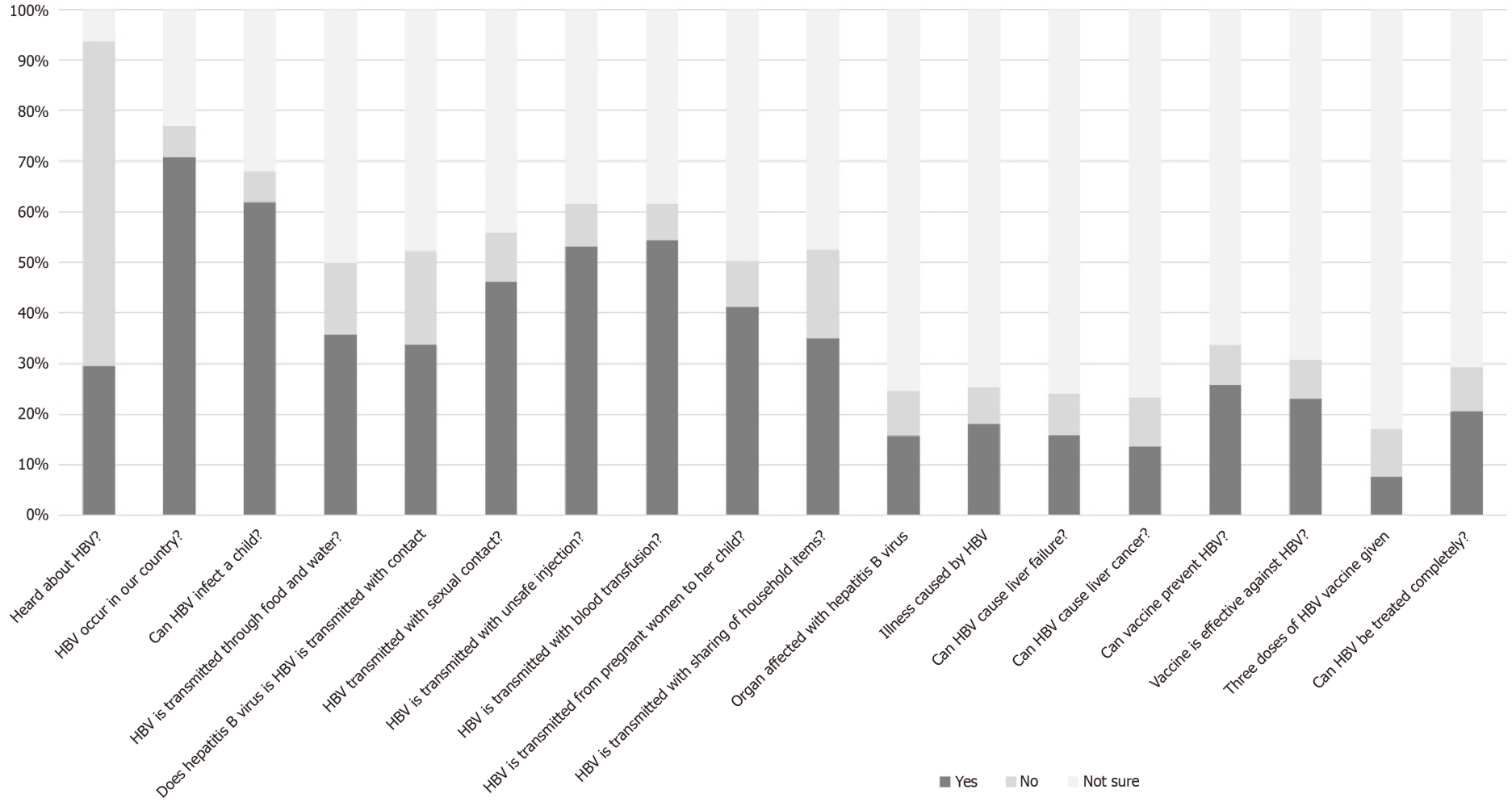

Figure 1 Summary of the responses given by the parents to the questions asked to assess their knowledge and awareness about hepatitis B virus. HBV: Hepatitis B virus.

national immunization schedule recommends Hep-BD within 24 h of birth followed by three doses of Hep-B3 at 6, 10 and 14 wk[7].

National Family Health Survey (NFHS)-5 reported that 77% and 70% of 12-23 months old children in the country and the state of Uttar Pradesh respectively, had received complete basic vaccination. In our study, 82% children were age-appropriately vaccinated. Our proportion was slightly higher than the national and state average because 78% were from

Table 1 Characteristics of the children and their parents included in study (*n* = 384)

Character	Value
Gender of the child (boys)	226 (58.9)
Age (months)	
≤ 18	167 (43.5)
19-59	175 (45.6)
≥ 60	42 (10.9)
Birth order	
First	208 (54.2)
Second	116 (30.2)
Third	38 (9.9)
Fourth	17 (4.4)
Fifth	5 (1.3)
Place of delivery	
Home delivery	22 (5.7)
Hospital delivery	362 (94.3)
Religion	
Hindu	200 (52.1)
Muslim	181 (47.1)
Other religion	3 (0.8)
Residence	
Rural	83 (21.6)
Urban	301 (78.4)
Information provided by	
Mother	224 (58.3)
Father	135 (35.2)
Any other guardian	25 (6.5)
Education of mother	
Illiterate	76 (19.8)
Up to class 8th	65 (16.9)
Class 9th to class 12th	106 (27.6)
Graduate	108 (28.1)
Postgraduate or professional	29 (7.6)
Education of father	
Illiterate	52 (13.5)
Up to class 8th	78 (20.3)
Class 9th to class 12th	98 (25.5)
Graduate	109 (28.4)
Postgraduate or professional	47 (12.2)
Setting in which vaccination were primarily done	
Public hospital	331 (86.2)
Private hospital	53 (13.8)
Parents perception about the vaccines	

Vaccine is a nutritional supplement	
Yes	10 (2.6)
No	317 (82.6)
Not sure	57 (14.8)
Vaccine is given to promote growth	
Yes	20 (5.2)
No	311 (81)
Not sure	53 (13.8)
Vaccine is given to prevent infections	
Yes	359 (93.5)
No	4 (1)
Not sure	21 (5.5)

Categorical data are expressed as *n* (%).

urban background, 50% were first child of the family, 58% were boys, and only 20% mothers were illiterate. The NFHS-5 report suggests that these factors are associated with better vaccination coverage in the country[8].

We found that the parents had limited knowledge and awareness about the HBV, its transmission, illness caused by the virus, methods of HBV prevention, and its treatment. Poor awareness of the parents may be one of the reasons for the inadequate coverage for Hep-BD and Hep-B3 doses in the country. The NFHS-5 survey reported that only 67% and 84% of 12-23 months aged children were given Hep-BD and Hep-B3 in our country. The Hep-BD and Hep-B3 coverage was even lower in the state of Uttar Pradesh and stood at 50% and 78% respectively[8]. The national and state coverage of Hep-BD and Hep-B3 are way below the target of > 90%, which is to be achieved in a short period of next seven years, *i.e*, 2030.

Efforts are being made at national and international levels to identify the risk factors for incomplete vaccination so that appropriate corrective measures can be taken to improve the vaccination coverage. Poor knowledge and attitude of the parents towards childhood vaccination had been identified as a global issue, particularly in resource constraint developing countries. A recent systematic review identified that 22% of 838 reasons for under-vaccination was linked to parents' knowledge and attitude towards the vaccination; similarly, 42% of 19 reasons for non-vaccination were liked to parents' knowledge about vaccination[9]. Other factors were related to immunization systems, family characteristics, and communication and inadequate information. The authors further concluded from the data that under-vaccination and non-vaccination due to parental attitudes and knowledge are more difficult to address.

The risk factors for incomplete childhood vaccination in Indian community, are non-institutional delivery[10], female gender, muslim religion, lower caste, fewer antenatal care visits, non-receipt of maternal tetanus vaccination, education status of the mother[11], and poor financial status of the family. None of the studies from India had exclusively focused on parents' knowledge about hepatitis B vaccine. Though, another study from Pakistan has also revealed that parents' knowledge and unawareness is a risk factor for the poor coverage of hepatitis B vaccine[12].

A large proportion of parents had lack of knowledge and awareness about hepatitis. Multiple studies have reported the low knowledge score about hepatitis B among parents[13], pregnant women[14], general population[15], as well as medical students[16]. We found that the parents of the children who were given Hep-B3, had significantly more knowledge and awareness about hepatitis B than their counterparts of children without Hep-B3. Another study from India has concluded that interventions to improve the knowledge of parents about vaccination have potential to improve the vaccination overage in India[17]. We understand that this association of parents' knowledge with hepatitis B vaccination may be an effect instead of the cause for the same. We need to explore this association in future studies.

Our study is the first study from the country which exclusively focused on hepatitis B vaccine and evaluated the knowledge and awareness of the parents about hepatitis B. Our study had a reasonable sample size and included children of different age groups. On the other hand, our study lacked a control arm to compare our results. We need to validate our results in multicentric studies before intervention can be implemented.

CONCLUSION

Coverage of Hep-BD and Hep-B3 vaccine is inadequate in the country. The parents have inadequate knowledge and awareness about hepatitis-B which may be partially responsible for poor vaccination coverage in their children. Measures shall be taken to raise the level of knowledge and awareness of the parents which could results in improved hepatitis B vaccination coverage in the country and may reduce HBV related morbidity and mortality in long term.

Table 2 Awareness of the parents about the vaccines included in childhood immunization program

Vernacular name of the childhood illness	Response of the parents	
	Yes	No
Tuberculosis	51.8	48.2
Poliomyelitis	94.3	5.7
Hepatitis B virus	29.2	70.8
Diphtheria (Gal Ghotu)	20	80
Pertussis (Kali Khanshi)	7.8	92.2
Measles (Khasara)	68	32
Mumps (Galsua)	29.7	70.3
Pneumonia	69	31
Tetanus	87.5	12.5
Rubella	10.2	89.8
Diarrhoea	70	30
Meningitis/Encephalitis (Dimagi Bukhar)	56.5	43.4

Data are presented as percentage (%).

Table 3 Knowledge and awareness of the parents about the hepatitis B (*n* = 384)

Domain examined	No.	Question	Response of the parents			Domain performance
			Yes	No	Not sure	
Epidemiology	1	Have you heard about hepatitis B vaccine?	29.5	64.2	6.3	61.9
	2	Does hepatitis B virus infection occur in our country?	70.8	6.2	23	
	3	Can hepatitis B virus infect a child?	61.9	6.0	32.1	
Route of transmission	4	Does hepatitis B virus transmit through food and water?	35.8	14.1	50.1	41.2
	5	Does hepatitis B virus transmit with casual contacts such as playing, handshake *etc*.?	33.7	18.5	47.8	
	6	Does hepatitis B virus transmit with sexual contact?	46.2	9.7	44.1	
	7	Does hepatitis B virus transmit with unsafe injection?	53.2	8.4	38.4	
	8	Does hepatitis B virus transmit with blood transfusion?	54.3	7.3	38.4	
	9	Does hepatitis B virus transmit from pregnant women to her child?	41.2	9.1	49.7	
	10	Does hepatitis B virus transmit with sharing of food, bed, utensils *etc*.?	35.0	17.5	47.5	
Consequences of hepatitis B virus infection	11	Which organ is affected with hepatitis B virus?	15.7	8.9	75.4	15.8
	12	What illness is caused by hepatitis B virus? (correct answer was Jaundice or similar phrases)	18.0	7.3	74.7	
	13	Can hepatitis B virus cause liver failure?	15.9	8.1	76.0	
	14	Can hepatitis B virus cause liver cancer?	13.6	9.7	76.7	
Prevention of HBV infection	15	Is there any vaccine to prevent hepatitis B infection?	25.8	7.8	66.4	23.0
	16	Is the vaccine effective against hepatitis B infection?	23.0	7.8	69.2	
	17	How many doses of hepatitis B vaccine are given in routine vaccination? (correct answer is three dose)	7.6	9.4	83.0	
Treatment of hepatitis B	18	Can hepatitis B be treated completely?	20.6	8.6	70.7	70.7

Data are expressed as percentage (%).

Table 4 Comparison of children with or without age-appropriate hepatitis B vaccination

Character	Values		*P* value
	Child is vaccinated for hepatitis B (*n* = 322)	Child is not vaccinated for hepatitis B (*n* = 62)	
Gender of the child (boys)	59	58	0.89
Age (months)			0.548
≤ 18	37	73	
19-59	51	18	
≥ 60	11	9	
Birth order			0.4
First	56	45	
Second	29	39	
Third	10	11	
Fourth	4	5	
Fifth	1	0	
Place of delivery			< 0.01
Home delivery	4	13	
Hospital delivery	96	87	
Religion			0.08
Hindu	54	40	
Muslim	45	60	
Other religion	1	0	
Residence			0.14
Rural	23	14	
Urban	77	86	
Education of mother			0.13
Illiterate	20	16	
Up to class 8th	15	24	
Class 9th to class 12th	28	26	
Graduate	30	21	
Postgraduate or professional	7	13	
Education of father			0.32
Illiterate	14	13	
Up to class 8th	19	26	
Class 9th to class 12th	26	22	
Graduate	30	21	
Postgraduate or professional	11	18	
Setting in which vaccination were primarily done			< 0.01
Public hospital	73	89	

Private hospital	27	11	
Any family member has or had hepatitis B infection			0.36
Yes	2	3	
No	90	84	
Not sure	8	13	

Data are presented as percentage (%).

Table 5 Comparison of hepatitis B related knowledge and awareness of parents of children with or without hepatitis B vaccination

No.	Question related to hepatitis B knowledge and awareness	Parents' response	Childs vaccination status		*P* value
			Child is vaccinated for hepatitis B (*n* = 322)	Child is not vaccinated for hepatitis B (*n* = 62)	
1	Have you heard about hepatitis B vaccine?	Yes	29	36	0.39
		No	65	56	
		Not sure	6	8	
2	Does hepatitis B virus infection occur in our country?	Yes	73	58	0.02
		No	5	13	
		Not sure	22	29	
3	Can hepatitis B virus infect a child?	Yes	65	47	< 0.01
		No	4	16	
		Not sure	31	37	
4	Does hepatitis B virus transmit through food and water?	Yes	38	24	0.04
		No	13	23	
		Not sure	49	53	
5	Does hepatitis B virus transmit with casual contacts such as playing, handshake *etc.*?	Yes	37	19	< 0.01
		No	16	32	
		Not sure	47	49	
6	Does hepatitis B virus transmit with sexual contact?	Yes	49	32	< 0.01
		No	8	21	
		Not sure	43	47	
7	Does hepatitis B virus transmit with unsafe injection?	Yes	55	47	0.01
		No	6	18	
		Not sure	39	35	
8	Does hepatitis B virus transmit with blood transfusion?	Yes	56	43	< 0.01
		No	6	18	
		Not sure	38	39	
9	Does hepatitis B virus transmit from pregnant women to her child?	Yes	44	29	0.01
		No	7	18	
		Not sure	49	53	
10	Does hepatitis B virus transmit with sharing of food, bed, utensils *etc.*?	Yes	38	21	0.02
		No	16	27	
		Not sure	46	52	

11	Which organ is affected with hepatitis B virus?	Yes	15	23	0.02
		No	7	16	
		Not sure	78	61	
12	What illness is caused by hepatitis B virus? (answer was Jaundice or similar phrases)	Yes	17	23	< 0.01
		No	6	18	
		Not sure	77	59	
13	Does hepatitis B virus cause liver failure?	Yes	15	19	< 0.01
		No	6	19	
		Not sure	79	62	
14	Does hepatitis B virus cause liver cancer?	Yes	13	18	< 0.01
		No	7	22	
		Not sure	80	60	
15	Is there any vaccine to prevent hepatitis B infection?	Yes	26	27	< 0.01
		No	6	18	
		Not sure	68	55	
16	Is the vaccine effective to prevent hepatitis B infection?	Yes	23	23	< 0.01
		No	6	18	
		Not sure	71	59	
17	How many doses of hepatitis B vaccine are given in routine vaccination?	Yes	20	23	< 0.01
		No	6	21	
		Not sure	74	56	
18	Can hepatitis B be treated completely?	Yes	8	6	< 0.01
		No	7	21	
		Not sure	85	73	

Data are presented as percentage (%).

FOOTNOTES

Author contributions: Nanda C, Srivastava G, Waseem M recruited the participants, collected data, analyzed data and prepared the first draft of manuscript; Yadav A, Singh S, Singh R, Goel A conceptualized the idea, designed the study, reviewed and edited the manuscript.

Institutional review board statement: Study was approved by the institute ethic committee (Approval No. ELMC & H/R_Cell/EC/2020/20).

Informed consent statement: All subjects understood and agreed to the study protocol and voluntarily signed the informed consent form.

Conflict-of-interest statement: Dr. Amit has nothing to disclose.

Data sharing statement: Raw data will be available with the corresponding authors and the same could be provided, after securing institute ethic committee approval, on a logical request.

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

Country of origin: India

ORCID number: Amit Goel 0000-0003-3525-9381.

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ORIGINAL ARTICLE

Randomized Clinical Trial

Hydroxychloroquine-azithromycin, doubase C, and QTc prolongation in congolese patients with COVID-19: Myth or reality?

Brady Madioko Makanzu, Jean-Robert Makulo, Madone Ndona Mandina, Dimosi Roger Wumba, Murielle Mashi Longokolo, Hippolyte Situakibanza, Ossam Odio, Donat Sonzi Mangala, Constantin Mihigo Bashengezi, Benjamin Kabwe Mwilambwe, Gilbert Kabanda Kurhenga, Benjamin Longo-Mbenza, Roger Mwimba Mbungu

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Brady Madioko Makanzu, Gilbert Kabanda Kurhenga, Benjamin Longo-Mbenza, Department of Cardiology, Kinshasa University Hospital, Kinshasa, DR Congo, Kinshasa University Hospital, Kinshasa 11, Congo

Jean-Robert Makulo, Nephrology Unit, Kinshasa University Hospital, Kinshasa, Democratic Republic of the Congo, Kinshasa 11, Kinshasa, Congo

Madone Ndona Mandina, Ossam Odio, Department of Infectious Diseases, Kinshasa University Hospital, Kinshasa, DR Congo, Kinshasa 11, Congo

Dimosi Roger Wumba, Department of Tropical Medicine, University of Kinshasa, Kinshasa 11, Congo

Murielle Mashi Longokolo, Department of Infectious Diseases, University of Kinshasa Hospital, Kinshasa 11, Congo

Hippolyte Situakibanza, Department of Tropical Medicine and Infectious Disease, School of Medicine, University of Kinshasa, Kinshasa 11, Congo

Donat Sonzi Mangala, COVID-19 Treatment Center, Kinshasa University Hospital, Kinshasa, Kinshasa 11, Congo

Constantin Mihigo Bashengezi, Centre de Recherche en Phytothérapie, Pharmacopée Africaine et Technologie pharmaceutique, Kinshasa 11, Congo

Benjamin Kabwe Mwilambwe, Intensive Care Unit, Kinshasa University Hospital, Kinshasa 11, Congo

Roger Mwimba Mbungu, Department of Gynecology and Obstetrics, Kinshasa University Hospital, Kinshasa 11, Congo

Corresponding author: Brady Madioko Makanzu, MD, Doctor, Department of Cardiology, Kinshasa University Hospital, Kimwenza Road, Lemba Municipality, Kinshasa 11, Congo. bmaknzu@gmail.com

Abstract

BACKGROUND

QTc interval prolongation with an increased risk of torsade de pointes (Tsd) has been described in coronavirus disease 2019 (COVID-19) patients treated with hydroxychloroquine (HCQ) and azithromycin (AZI) in Western countries. In the DR Congo, few studies have evaluated the safety of this association or proposed new molecules.

AIM

To determine the incidence of QTc prolongation and Tsd in COVID-19 patients treated with HCQ-AZIs *vs* doubase C (new molecule).

METHODS

In present randomized clinical trial, we have included patients with mild or moderate COVID-19 treated with either HCQ-AZI or doubase C. Electrocardiogram (ECG) changes on day 14 of randomization were determined based on pretreatment tracing. Prolonged QTc was defined as ≥ 500 ms on day 14 or an increase of ≥ 80 ms compared to pretreatment tracing. Patients with cardiac disease, those undergoing other treatments likely to prolong QTc, and those with disturbed ECG tracings were excluded from the study.

RESULTS

The study included 258 patients (mean age 41 ± 15 years; 52% men; 3.4% diabetics, 11.1% hypertensive). Mild and moderate COVID-19 were found in 93.5% and 6.5% of patients, respectively. At baseline, all patients had normal sinus rhythm, a mean heart rate 78 ± 13/min, mean PR space 170 ± 28 ms, mean QRS 76 ± 13 ms, and mean QTc 405 ± 30 ms. No complaints suggesting cardiac involvement were reported during or after treatment. Only four patients (1.5%) experienced QTc interval prolongation beyond 500 ms. Similarly, only five patients (1.9%) had an increase in the QTc interval of more than 80 ms. QTc prolongation was more significant in younger patients, those with high viral load at baseline, and those receiving HCQ-AZI ($P < 0.05$). None of the patients developed Tsd.

CONCLUSION

QTc prolongation without Tsd was observed at a lower frequency in patients treated with HCQ-AZI *vs* doubase C. The absence of comorbidities and concurrent use of other products that are likely to cause arrhythmia may explain our results.

Key Words: QTc prolongation; COVID-19; Doubase C; HCQ-AZT; Sub-Saharan Africa

Core Tip: During the coronavirus disease 2019 (COVID-19) pandemic, western studies have shown the ineffectiveness and cardiotoxic effects of hydroxychloriquine and azithromycin (HCQ-AZI). In Africa, the heart toxicity of HCQ-AZI is little reported by cardiologists, and some African countries used these two molecules to treat COVID-19 patients. In the present study, we have evaluated the occurrence of prolongation of the QTc interval and torsade de pointes torsade in Congolese COVID-19 patients who received HCQ-AZI compared to doubase C, a herbal medicine with broad-spectrum antiviral activity.

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INTRODUCTION

The first coronavirus disease 2019 (COVID-19) cases were reported in Wuhan, China in December 2019[1]. COVID-19 then spread very quickly across the world and was declared a pandemic on March 11, 2020 by the World Health Organization (WHO)[2]. COVID-19 curative treatment has been controversial. Some countries, particularly in Africa, have used hydroxychloroquine (HCQ) or chloroquine (CQ) alone or in combination with azithromycin (AZI) without much solid evidence; however, in general, COVID-19-related mortality in Africa was not as high as in countries that did not use this treatment[3].

In addition to adverse effects such as allergies and retinal damage, HCQ and CQ can cause cardiac arrhythmia and prolong the QTc interval[4-8]. This risk of QTc prolongation increases when AZI is added to the treatment and can potentially develop into torsade de pointes (Tsd), thus increasing patient mortality[4-8]. Series published in the West, including that of Bessière *et al*[9] in France, found that more than 35% of patients had a prolongation of the QTc interval. Healthcare teams have raised concerns about the use of HCQ and AZI. Therefore, electrocardiogram (ECG) must be

monitored regularly during treatment[10]. In several hospitals in the West, HCQ was no longer used in the treatment of COVID-19 patients[11].

Although the cardiac side effects of HCQ/CQ have been reported in patients with COVID-19 in Western countries, few studies have been conducted in sub-Saharan Africa (SSA), particularly in the DR Congo, where HCQ/CQ has been previously used to treat malaria or common rheumatological diseases such as lupus erythematosus and rheumatoid arthritis. This is also the case for other molecules used to treat severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection during the COVID-19 pandemic in SSA. However, their efficacy and safety have not been evaluated within the population. In 2021, a randomized clinical trial was conducted in Kinshasa to compare the efficacy and safety of HCQ-AZI versus doubase C, a plant-based medicine, in the treatment of COVID-19[12]. We assessed the risk of cardiac toxicity associated with these two molecules because we did not have local data.

This clinical trial aimed to determine the incidence of QTc prolongation, its predictors and the consequences (Tsd and other arrhythmia) in COVID-19 patients (mild and moderate cases) followed at Kinshasa University Hospital (KUH).

MATERIALS AND METHODS

Study design

Several aspects of the general methodology of this study have been published in the WHO Clinical Trials Registry[12]. We collected data from the KUH COVID-19 treatment center (CTCO) and focused on assessing the cardiac safety of the two treatments from May 2021 to January 2022.

Doubase C is a large-spectrum antiviral derived from two plants, *Uvaria brevistipita* and *Haroungana madasgascariensis*. The treatment was given over 7 d depending on body weight: 2 tablets 3 times per day if weight < 80 kg; 3 tablets 3 times a day between 80-99 kg and 4 tablets 3 times a day if weight ≥ 100 kg[12].

At the start of the pandemic in DR Congo, HCQ-AZI was the reference protocol: HCQ was prescribed at a dose of 200 mg 3 times a day for 10 d while AZI was given over 5 d, *i.e.* 500 mg the first day, then 250 mg for 4 d[12,13]. In addition to the basic treatment, all patients received zinc sulfate (20 mg once a day), vitamin C (500 mg once a day) and vitamin D (400 IU three times per day) for 10 d. Diabetic and hypertensive patients continued their usual treatment. Oxygen was indicated when O2pSa was less than 95%[13].

Sampling

Inclusion criteria: Patients had to be at least 18 years old, have mild or moderate COVID-19, sign informed consent, receive one of two medications according to the clinical trial protocol, and have an ECG examination before the treatment, and then after 7 and 14 d.

Exclusion criteria: Pregnant women; patients with cardiac, kidney, or liver disease; asymptomatic, severe, or critical COVID-19; and all patients who had a prolonged QTc interval on ECG before treatment.

Patients who presented with complications (hematological, renal, hepatic, cardiac, or neurological) left the study and were treated in the hospital. In the event of prolongation of the QTc interval during the study, treatment was immediately stopped, and the patient was followed up in the Cardiology Department.

Sample collection

Parameters unrelated to this study are described in detail elsewhere[12]. For this study, we used the following variables of interest: age, sex, history of diabetes, hypertension, current treatment, COVID-19 treatment, vital signs at each visit, days 1, 7, and 14 of randomization (consciousness, blood pressure, heart and respiratory rates, and temperature), oxygen saturation, and ECG results (on days 1 and 14).

Electrocardiogram: The MAC ECG 600 device made it possible to perform examinations with twelve leads at a speed of 25 mm/s. The protocols were carried out by two cardiologists in a consensual manner. The device gave measurements of the QTc, QT, QRS and PR intervals. Lead D2 or V5 was used to calculate the QTc interval. When the heart rate fluctuated between 60 and 100 beats/min, the Bazett formula was applied and when it was less than 60 beats/min or more than 100 beats/min, the Fredericia formula was used[14]. In case of bundle branch block, the JTC formula: [QT- (QRS-120 ms)]/$\sqrt{RR}$ was used[15].

Operational definitions

The clinical form of COVID-19 was based on the WHO classification[16]. The baseline SARS-CoV-2 viral load was determined using the CTE and CTN2[17]. The QTc (lead D2 or V5) was defined as the duration from the beginning of the QRS complex to the end of the T-wave upon its return to baseline. Prolonged QTc was defined as QTc interval ≥ 470 ms for women and ≥ 450 ms for men[14]. QTc requiring discontinuation of treatment was defined by a prolonged QTc ≥ 500 ms[8] or by a QTc increase > 80 ms[18].

Endpoint

The endpoint was ECG change on day 14 of randomization (QTc ≥ 500 ms or QTc increase > 80 ms).

Ethics approval

The present study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Kinshasa School of Public Health (ESP/CE/038/2021). All data were fully anonymized before being accessed.

Statistical analyses

After verification, the data were analyzed using SPSS Statistics version 25.0. Categorical variables are presented as absolute and relative frequencies (%) while continuous variables are presented as mean ± SD. The Kolmogorov-Smirnov test was used to check whether the data distribution was Gaussian. The Student's *t* test was used to compare the means between two groups. The MacNemar test was used to compare paired data. The statistical significance threshold was set at $P < 0.05$.

RESULTS

Baseline characteristics of patients

261 patients were randomly assigned to two arms: 123 (47%) were treated with HCQ-AZI and 138 (53%) with doubase C. Among them, 93.5% of patients had mild and 6.5% had moderate COVID-19. The mean patient age was 41 ± 15 years and the sex ratio was 1.1. The average patient BMI was 26.1 ± 5.4 Kg/m², and the average partial oxygen saturation was 96.9 ± 2.0%. Patients with a medical history of hypertension, diabetes, HIV/AIDS, asthma, or tuberculosis accounted for 11.1%, 3.4%, 0.4%, 2.3%, and 0.4%, respectively. The averages of hemoglobin (13.6 ± 2.7 g/dL), creatinine (0.97 ± 0.30 mg/dL), AST (28 ± 12 IU/L) and ALT (25 ± 16 IU/L), were not statistically different between the two arms (Table 1).

Table 2 shows that the various ECG parameters were normal at the initiation of treatment. The mean QTc was 405 ± 30 ms.

Patient complaints and changes in ECG pattern during treatment

During treatment, none of the patients experienced disease worsening. No patient was admitted to the intensive care unit and none died. Only three patients did not undergo ECG on day 14. They preferred not to attend follow-up meetings because they felt well.

Among the 258 patients who visited on the 14th-day appointment, none of the complaints suggesting cardiac involvement, such as dyspnea, shortness of breath, palpitations, lipotymia, or sudden death, were reported. Table 3 shows that the mean QTc interval increased from 411 ± 41 to 418 ± 37 ms and the PR space from 169 ± 25 to 177 ± 3.6 ms.

Table 4 shows that only four patients (1.5%), all from the HCQ-AZI group, presented a prolongation of the QTc interval beyond 500 ms. Similarly, only five patients (1.9 %) in the HCQ-AZI arm had a more than 80 ms increase in the QTc interval (Table 4).

As shown in Table 5, QTc interval prolongation was greater in younger patients, those with a high viral load, and those who received HCQ-AZI. No cases of Tsd were observed throughout the study.

DISCUSSION

QTc interval prolongation under HCQ is linked to the blockade of the HERG potassium channel, which is involved in the final phase of repolarization. HERG channel blockade prolongs ventricular repolarization and can lead to Tsd[19,20]. HCQ can also act on sinus nodes and cause bradycardia[20]. Macrolide antibiotics such as AZI block the HERG channel [21]. In the present study, a few patients treated with HCQ-AZIs experienced QTc interval prolongation; however, the incidence was significantly lower than that in studies conducted in Western countries[6-9], and the abnormality was not associated with cardiac complications. A meta-analysis that selected 13 studies reported incidences of QTc interval prolongation varying between 0 and 35% in patients with COVID-19 who received HCQ or CQ in combination or not with AZI[22]. Six studies were conducted in France, four in the United States, one in Italy, one in Holland, and one in Brazil. The authors did not report the proportion of Black patients in these studies.

Several factors may explain the differences between studies, such as the threshold used to define QTc interval prolongation (≥ 500 ms, increase of 60 ms or 80 ms compared to the baseline value, and other different thresholds), the clinical state of the patients (critical patients in intensive care units or patients with less severe disease), and the concomitant use of other medications likely to prolong the QTc interval. In addition to medications, electrolyte disorders, including hypokalemia and hypomagnesemia, are the most frequently encountered risk factors. The role of hypocalcemia is less obvious, as low serum calcium levels prolong the QTc interval[23]. Cardiac disease and acute stroke are also risk factors[23]. The absence of major comorbidities, severe and critical forms of COVID-19, concurrent use of other medications, and lower doses could explain the low incidence of QTc prolongation in our patients treated with HCQ-AZIs.

The absence of major cardiac complications such as Tsd with HCQ-AZI is consistent with incidence varying between 0 and 1.11%[22]. Studies reporting cases of sudden death deserve to be reevaluated because they included intensive care patients who presented with several comorbidities and were administered very high doses of HCQ[6-9]. Our study is, to the best of our knowledge, the only study to have evaluated the safety of doubase C. Being a new drug, studies with larger sample sizes must be conducted to confirm that the product does not prolong the QTc interval and has no risk of

Table 1 Baseline characteristics of patients

Variables	Whole group, *n* = 261	Doubase C, *n* = 138	HCQ-AZI, *n* = 123	*P* value
Age	41.0 ± 14.8	40.5 ± 15.3	41.5 ± 14.2	0.581
Men/women	137/134	64/74	73/50	0.036
BMI	26.1 ± 5.4	26.4 ± 5.7	25.6 ± 5.0	0.249
O2pSa	96.9 ± 2.0	97.0 ± 1.7	96.9 ± 2.3	0.449
Hypertension	29 (11.1)	18 (13.0)	11 (8.9)	0.569
Diabetes	9 (3.4)	7 (5.1)	2 (1.6)	0.197
HIV	1 (0.4)	1 (0.7)	0	0.386
Asthma	6 (2.3)	3 (2.2)	3 (2.4)	0.744
Tuberculosis	1 (0.4)	1 (0.7)	0	0.383
Hemoglobin (g/dL)	13 ± 3	13.7 ± 3.2	13.5 ± 2.2	0.673
Creatinine (mg/dL)	0.97 ± 0.30	0.96 ± 0.31	0.99 ± 0.25	0.420
ALAT (UI/L)	25 ± 16	23 ± 14	27 ± 18	0.064
ASAT (UI/L)	28 ±12	29 ± 14	26 ± 11	0.141
K^+ (mmol/L)	4.2 ± 2.0	3.9 ± 1.0	3.8 ± 0.11	0.167
Ca^{2+} (meq/L)	1.17 ± 0.11	1.15 ± 0.11	1.18 ± 0.11	0.430

The results are presented as means and standard deviations or as absolute frequencies (percentages). BMI: Body mass index; HIV: Human immunodeficiency virus; ALAT: Alanine aminotransferase; ASAT: Aspartate aminotransferase; HCQ-AZI: Hydroxychloriquine and azithromycin.

Table 2 Baseline characteristics of electrocardiograms

ECG parameter	Participants, *n* = 261	Doubase C, *n* = 138	HCQ-AZI, *n* = 123	*P* value
Sinus rhythm	261 (100)	123 (100)	138 (100)	
Heart rate (/min)	78 ± 13	79 ± 13	77 ± 13	0.420
P wave (ms)	114 ± 10	113 ± 12	115 ± 8	0.495
PR space (ms)	170 ± 28	172 ± 30	168 ± 26	0.331
QRS complex (ms)	76 ± 13	76 ± 14	76 ± 12	0.839
T-wave (ms)	193 ± 32	193 ± 30	194 ± 34	0.679
QTc interval (ms)	405 ± 30	406 ± 31	401 ± 29	0.193
Sokolow- Lyon (mm)	24.5 ± 8.9	25.0 ± 10.1	23.1 ± 8.0	0.611

HCQ-AZI: Hydroxychloriquine and azithromycin.

Tsd.

In addition to the HCQ-AZI combination, viral load and age were associated with QTc interval prolongation. The signs of a prolonged QTc interval can occur under specific conditions such as exercise, stress, emotion, or auditory stimulation [24]. In hindsight, we know that there are no cardiac arrhythmias specific to COVID-19, but a particular discrepancy between heart rate and temperature can be observed in a few patients. Therefore, the heart rate may be slower than expected in relation to temperature[25]. This phenomenon has also been observed in other infectious diseases such as typhoid fever. Hypoxemia can also play a role in explaining arrhythmias. With regard to age, we should expect a much higher QTc frequency in elderly patients than in patients aged 10 and 30 years, as observed for certain congenital forms of prolonged QTc[24].

Despite the interest in this study, some limitations are worth mentioning. ECG was not performed daily, which may have reduced the frequency of detected abnormalities. In some studies, the ECG was performed on the day after drug administration. In our study, for doubase C, after 7 d of administration of the last dose; in the HCQ-AZI arm, HCQ was taken from days 1 to 10, AZI from days 1 to 5, and the ECG check was performed on day 14. However, HCQ has a long duration of action, as it may be taken weekly for some indications[26]. Indeed, at a dose of 200 mg, the plasma half-life of HCQ is 30 d[24]. The half-life of AZI is shorter at, 2-4 d[27]. The small sample size is also a limitation of this study.

Table 3 Electrocardiogram changes after treatment (day 14 of randomization)

Variable	Days 14-1	Day 1	Day 14	*P* value
Heart rate (/min)	0.820	78 ± 13	79 ± 12	0.343
P wave (ms)	1.010	114 ± 10	115 ± 13	0.314
PR space (ms)	8.232	170 ± 28	177 ± 36	0.001
QRS complex (ms)	0.469	76 ± 13	76 ± 13	0.560
QTc interval (ms)	8.835	405 ± 30	413 ± 35	< 0.001
T-wave (ms)	3.867	193 ± 32	197 ± 42	0.197
Sokolow–Lyon (mm)	0.004	24.5 ± 8.9	24.5 ± 8.5	0.990

Table 4 Proportion of patients who experienced prolongation of QTc after treatment

Treatment	QTc, ≥ 500 ms		QTc, < 500 ms		*P* value	QTc increase, 80 ms		No QTc irncrease		*P* value
	n	%	*n*	%		*n*	%	*n*	%	
HCQ-AZI	4	3.3	117	96.7	0.047	5	4.1	116	95.9	0.021
Doubase C	0	0	137	100		0	0	137	100	

HCQ-AZI: Hydroxychloriquine and azithromycin.

Table 5 Risk of electrocardiogram pattern changes (QTc prolongation) in each subgroup

Variables	QTc prolongation		*P* value
	Day 1, *n* = 261	Day 14, *n* = 258	
Men	0	1	0.500
Women	0	4	0.061
Age < 40 yr	0	5	0.031
Obesity	0	2	0.247
Normal and overweight	0	3	0.124
CtN2 or CtE < 33	0	5	0.030
HCQ-AZI	0	5	0.030

CtE: Cycle threshold value of envelope genes of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); CtN2: Cycle threshold value of nucleoprotein genes of SARS-CoV-2; HCQ-AZI: Hydroxychloriquine and azithromycin.

Regarding COVID-19 treatment in the two arms, the duration differed (7 and 10 d). However, this choice was made to comply with the two therapeutic strategies already in use in the country.

CONCLUSION

Contrary to studies published in Western countries, the risk of QTc interval prolongation was present in Congolese patients with COVID-19 and treated with the HCQ-AZI combination but at a low frequency and without Tsd. This risk was not observed for doubase C. The absence of comorbidities and the concomitant use of other products that are likely to cause arrhythmia may explain our results.

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FOOTNOTES

Author contributions: Makulo JR, Mandina MN, Makanzu BM, and Longo-Mbenza B conceptualized the research; Makanzu BM, Longokolo M, Odio O, Bepouka IB, and Mangala D screened patients and acquired clinical data; Makulo JR and Makanzu BM wrote the first verion of the paper; All the authors have read and approved the final manuscript; Kabanda KG applied for and obtained the funds for this research project; Makulo JR, Mandina MN, and Longo-Mbenza B supervised the whole process of the study.

Institutional review board statement: The present clinical trial is an adaptation of the standard protocol validated by the WHO for clinical trials of herbal medicines used in the treatment of COVID-19 (WHO Master Protocol Version number 1.0; 09 June 2020). The access to patients was granted by the DR Congo Ministry of Research (444/MIN.RSIT/CABMIN/JMK/2020). Our research projects on COVID-19 received the approval of the Ethics Committee of the Kinshasa School of Public Health of the University of Kinshasa (ESP/CE/038/2021).

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Country of origin: Congo

ORCID number: Brady Madioko Makanzu 0000-0001-9167-3822; Jean-Robert Makulo 0000-0001-5517-3281; Benjamin Longo-Mbenza 0000-0002-5164-6296.

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SYSTEMATIC REVIEWS

Exploring the impact of rotavirus vaccination on antibiotic prescription and resistance: A comprehensive systematic review

Lakshmi Venkata Simhachalam Kutikuppala, Matei-Alexandru Cozma, Gautam Maddineni, Harshal Prakash Chorya, Nayanika Tummala, Swathi Godugu, Jyothi Swaroop Chintala, Mihnea-Alexandru Găman

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Lakshmi Venkata Simhachalam Kutikuppala, Department of General Surgery, Dr NTR University of Health Sciences, Vijayawada 520008, Andhra Pradesh, India

Matei-Alexandru Cozma, Mihnea-Alexandru Găman, Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest 050474, Romania

Matei-Alexandru Cozma, Department of Gastroenterology, Colentina Clinical Hospital, Bucharest 020125, Romania

Gautam Maddineni, Department of Internal Medicine, Florida State University, Cape Coral, FL 33909, United States

Harshal Prakash Chorya, Department of Internal Medicine, Baroda Medical College, Vadodara 390001, Gujarat, India

Nayanika Tummala, Department of Internal Medicine, GITAM Institute of Medical Sciences and Research (GIMSR), Visakhapatnam 530045, Andhra Pradesh, India

Swathi Godugu, Department of Internal Medicine, Zaporozhye State Medical University, Zaporozhye 69000, Ukraine

Jyothi Swaroop Chintala, Department of Anesthesiology, Dr NTR University of Health Sciences, Vijayawada 520008, Andhra Pradesh, India

Mihnea-Alexandru Găman, Department of Hematology, Center of Hematology and Bone Marrow Transplantation, Fundeni Clinical Institute, Bucharest 022328, Romania

Corresponding author: Mihnea-Alexandru Găman, Doctor, MD, PhD, Doctor, Research Fellow, Researcher, Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy, No. 8 Eroii Sanitari Boulevard, Bucharest 050474, Romania. mihneagaman@yahoo.com

Abstract

BACKGROUND

Rotavirus is a highly contagious virus responsible for a significant burden of acute gastroenteritis, particularly among infants and young children worldwide, however, vaccination against this viral agent is available. Several studies have hypothesized that rotavirus vaccination has been linked to lower rates of antibiotic resistance.

AIM
To assess the relationship between rotavirus vaccination and antibiotic resistance.

METHODS
The present systematic review was tailored based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines. Several electronic databases (PubMed/MEDLINE, Scopus and Web of Science) were searched independently by two investigators in order to retrieve relevant publications published until April 2023 that investigated the aforementioned research question.

RESULTS
The comprehensive database search identified a total of 91 records. After the duplicates were removed (n = 75), we screened the titles and abstracts of 16 potentially eligible publications. After the irrelevant records were excluded (n = 5), we screened the full texts of 11 manuscripts. Finally, 5 studies were entered into the qualitative and quantitative analysis.

CONCLUSION
In conclusion, all the studies support the idea that vaccinations can reduce the need for antibiotic prescriptions which could potentially contribute to mitigating antibiotic resistance. However, to fully comprehend the mechanisms of antibiotic resistance, enhance treatment guidelines, and consider diverse demographic situations, further research is necessary to use evidence-based strategies to fight antibiotic misuse and resistance.

Key Words: Rotavirus; Vaccination; Gastroenteritis; Antibiotics; Antibiotic resistance; Diarrhea; Children

Core Tip: Vaccination against rotavirus has been hypothesized to reduce the need for antibiotic prescriptions. Herein, we conducted a systematic review to evaluate the relationship between antibiotic resistance and rotavirus vaccination. Our findings support the idea that vaccinations, including rotavirus vaccination, can reduce the need for antibiotic prescriptions which could potentially contribute to mitigating antibiotic resistance.

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INTRODUCTION

Rotavirus is a highly contagious virus responsible for a significant burden of acute gastroenteritis (AGE), particularly among infants and young children worldwide[1]. It is estimated that over 25 million emergency department visits and more than 2 million hospital admissions are attributed to rotavirus infections annually. According to the World Health Organization, in developed nations, a significant proportion of children (approximately 75%) experience the initial occurrence of rotavirus diarrhea before the age of 12 months. Conversely, in developing countries, the onset of the first episode of rotavirus diarrhea is often postponed until the age range of 2–5 years. The prevalence of severe rotavirus gastroenteritis is primarily observed in children between 6 months and 24 months of age[2]. From 2013 to 2017, approximately 122000–215000 infant deaths due to diarrhea were attributed to rotavirus each year[3-5]. Rotavirus has been identified as the third most prevalent infection linked to childhood mortality among children under 5 years of age[5]. Children residing in low- and medium-income countries (LMICs) have a disproportionate burden of diarrheal mortality compared to their counterparts in high-income countries[3].

Public health experts widely acknowledge the importance of rotavirus vaccination as a vital intervention to decrease the disease burden. The implementation of rotavirus vaccines has been associated with significant reductions in hospitalizations and fatalities caused by rotavirus in numerous countries[6]. Despite these positive outcomes, vaccination coverage for rotavirus varies greatly across different countries. It is crucial to explore the broader implications of this intervention, including its potential influence on antibiotic resistance. One such implication is the potential impact on antibiotic resistance. Antibiotic resistance, a growing public health concern, has emerged as a global challenge, impacting the effectiveness of bacterial infection treatments and outcomes[7,8]. While the association between viral infections and antibiotic resistance may seem counterintuitive, there is a need to investigate the potential relationship between rotavirus and antibiotic resistance to comprehend the broader implications for patient management, public health, and antimicrobial stewardship. The misuse and overuse of antibiotics have contributed significantly to the emergence and spread of antibiotic-resistant bacteria[8]. While rotavirus is a viral infection for which antibiotics are not typically indicated, it is

crucial to investigate if there are instances of antibiotic misuse in the management of rotavirus-related complications or co-infections.

Understanding the association between rotavirus and antibiotic resistance is essential due to the potential impact on patient outcomes[9]. There are several studies that show evidence of a decrease in antibiotic resistance with rotavirus vaccination. This potential effect is due to the decrease in the usage of antibiotics. One of the studies was a retrospective analysis that showed a decrease of the rotation of antibiotics needed in fully vaccinated children[7]. Other such studies showed decades worth of decrease in antibiotic resistance and usage in children who were vaccinated especially in low to middle-income countries[8-10]. Rotavirus infection can lead to compromised immune systems and damage to the intestinal lining, potentially increasing the susceptibility to secondary bacterial infections. In such cases, appropriate antibiotic therapy may be warranted, necessitating an assessment of potential antibiotic resistance patterns[10]. The association between rotavirus and antibiotic resistance may have implications for infection control strategies, especially in healthcare settings. Nosocomial infections pose a significant challenge, and if rotavirus-infected patients are more prone to acquiring bacterial infections, particularly those caused by antibiotic-resistant strains, it has implications for infection prevention and control measures[11].

This systematic review will provide a robust and evidence-based analysis of the existing literature on the association between rotavirus and antibiotic resistance. This systematic review aims to explore the association between rotavirus and antibiotic resistance, considering the implications for patient care, public health, and antimicrobial stewardship. By synthesizing the available evidence, we aim to shed light on this topic and provide insights that can inform clinical practice, policy-making, and future research endeavors in this field.

MATERIALS AND METHODS

The present systematic review was tailored based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (Table 1)[12]. The study protocol was registered in PROSPERO (International prospective register of systematic reviews) PROSPERO registration number CRD42023429481[13].

Several electronic databases (PubMed/MEDLINE, Scopus and Web of Science) were searched independently by two investigators in order to retrieve relevant publications published until April 2023. The search strategy was based on the use of keywords, word combinations and Medical Subject Headings, including: "Rotavirus Vaccination", "Rotaviral immunization", "Rotavirus", "Antibiotic resistance", "Antibiotic Prescribing", "Antimicrobial resistance", "Antibiotic prescribing", "Drug Resistance", "Antibiotics". In addition, we used the snowball strategy and checked the reference lists of all relevant manuscripts to avoid missing out any potentially eligible publications. No language restrictions were applied to the search.

We formulated the following Population Intervention Comparator Outcome (PICO) question: In children, does rotavirus vaccination reduce antibiotic prescribing from AGE or is it associated with a reduction in the growth of antibiotic resistance? The following PICO terms were used: (1) Population: Children who were born in areas with universal vaccination; (2) Intervention: Rotavirus vaccination; (3) Comparator: Non-vaccinated children; (4) Primary outcome: Reduction in antibiotic prescribing for AGE; and (5) Secondary outcomes: Reduction in growth of Antibiotic resistance.

We employed the following inclusion criteria: (1) Cross-sectional, cohort, and case-control, observational studies, as well as randomized and non-randomized clinical trials; (2) Studies conducted in children and individuals who were vaccinated against rotavirus; and (3) Papers published in languages spoken by the investigators (English, Hindi, French, Italian, Romanian). We excluded case reports, case series, narrative, scoping, and systematic reviews, meta-analyses, short communications (letters, commentaries), book chapters, study protocols, and conference abstracts, articles not published in languages spoken by the assessors, manuscripts with unavailable full-texts or published in non-peer reviewed journals, and investigations conducted in children with improper vaccination history and/or inadequately treated.

Rayyan software (Rayyan Systems Inc., Cambridge, MA) was employed to perform the screening of titles and abstracts. Relevant data was extracted in Google Sheets and analyzed using Microsoft Office Excel 2003. The following data were extracted and entered into a summarization table: Name of the first author of the study, year of publication of the study, country of execution, study type, study period, population, intervention, administration of other vaccinations, population of comparison, assessment for or history of infections, history of antibiotic use, antibiotic prescriptions, antibiotic prescription following AGE, and data on antimicrobial resistance. The quality of the included publications was evaluated using the Newcastle Ottawa Scale for observational studies and the Cochrane Risk of Bias (ROB) 2.0 tool for randomized controlled trials.

RESULTS

The systematic search of the databases identified a total of 91 records. After the duplicates were removed (n = 75), we screened the titles and abstracts of 16 potentially eligible publications. After the irrelevant records were excluded (n = 5), we screened the full-texts of 11 manuscripts. Finally, 5 studies were entered into the qualitative and quantitative analysis. The flowchart diagram of the literature search process is presented in Figure 1.

The characteristics of the publications entered in the systematic review are depicted in Table 1.

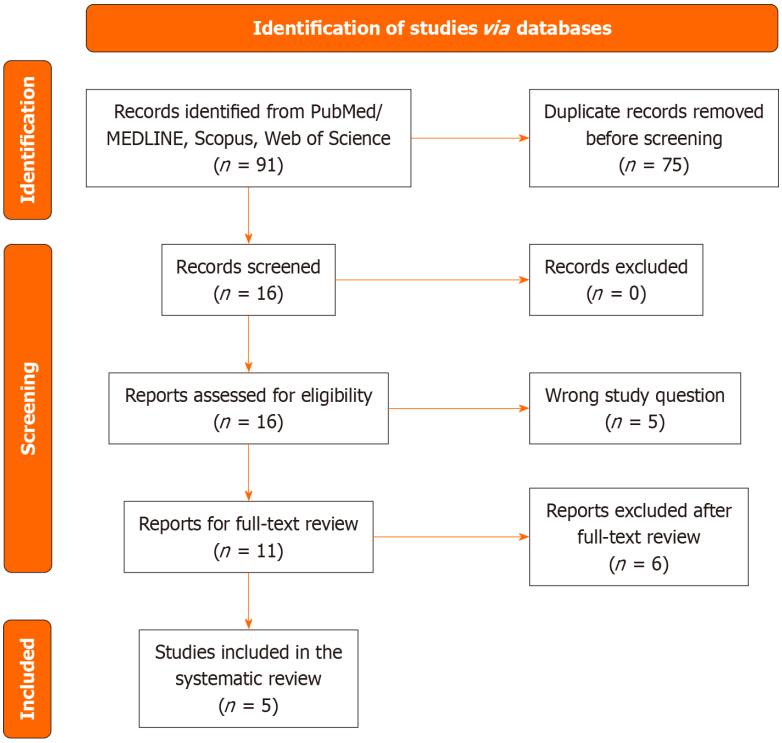

Figure 1 Preferred Reporting Items for Systematic reviews and Meta-Analyses flow diagram for the current systematic review.

The studies were published between 1990 and 2022 and conducted between 1985 and 2019 either in North America (United States of America, *n* = 1)[7], South America (Venezuela, *n* = 1)[14], Asia (Indonesia, *n* = 1)[15] or as multi-country international collaborations (Africa-Asia multicentric studies, *n* = 2)[16,17]. The research was designed as retrospective cohort studies (*n* = 1)[7], case-control studies (*n* = 2)[16,17] or clinical trial studies (*n* = 2)[14,15] and recruited participants of both sexes. The subjects analyzed were children aged 0 month to 60 months who did/did not experience diarrhea. The intervention explored consisted in the administration of the rotavirus vaccine alone[14,15] or in combination with other vaccines (*e.g.*, pneumococcal conjugate vaccine, *n* = 3)[7,16,17]. The comparator groups included children without diarrhea and/or who were not vaccinated against the rotavirus. Three studies assessed enrolled participants for medical history relevant for AGE and four manuscripts mentioned data on the antibiotic use of the analyzed children, however, the specific antibiotics/classes of antibiotics prescribed were not mentioned (Table 1). ROB analysis for the included studies showed high-quality of evidence for the majority (80%) of the evaluated observational studies (4 out of 5) (Table 2). Only one of the six included studies was randomized and its risk of bias was low according to the Cochrane ROB2 tool.

DISCUSSION

This systematic review comprehensively analyzes the intricate relationship between rotavirus and pneumococcal vaccinations in children, focusing on their impact on antibiotic prescriptions and broader implications for antibiotic resistance mitigation and the prevention of antibiotic-treated illnesses. The systematic exploration of the association between rotavirus vaccination and antibiotic resistance represents a critical endeavor in understanding the intricate dynamics between viral infections, antibiotic use, and resistance patterns. Our review aimed to dissect this relationship through an in-depth analysis of available literature, shedding light on potential implications for healthcare, antimicrobial stewardship, and public health strategies.

The retrieved studies, encompassing diverse geographical locations and study designs, provided valuable insights into this complex interplay. The results of case-control studies in several countries and retrospective cohort studies in the United States of America have different views on how the rotavirus vaccine might affect the use of antibiotics and the development of antibiotic resistance[7,16].

Children vaccinated against rotavirus experienced a significant decrease in the number of antibiotic prescriptions required after an AGE diagnosis, according to the study by Hall *et al*[7]. Rotavirus-vaccinated children with AGE had fewer antibiotic prescriptions and a lower likelihood of switching antibiotics within 28 d of their first prescription. This suggests that antibiotic resistance may be slowing down. The observed effect magnified over a 5-year follow-up, especially during the rotavirus season, bolstering cumulative vaccine effectiveness. Extrapolating these findings to the wider United States child population estimates a substantial prevention of over 67000 initial antibiotic prescriptions since the inception of the rotavirus vaccination[7].

Table 1 Characteristics of the publications included in the current systematic review

Ref.	Country	Study type	Study period	Population	Intervention	Other vaccinations (PCV, *etc.*)	Comparison	Assessment for (history of infections)	History of antibiotic use	Any antibiotic prescription	Antibiotic prescription following AGE	Antimicrobial resistance
Hall *et al* [7], 2022	Unite States	Retrospective cohort study	2007-2018	Children aged 5 born 2007-2018	RV	PCV	Children with no RV	AGE	Aminoglycosides, cephalosporin, β-lactam, erythromycin and macrolide, penicillins, miscel-laneous antibiotics, quinolones, sulfon-amides and combination, sulfones	NS	55.4% received antibiotics during the follow-up period; 1.5% of antibiotic prescriptions followed an AGE diagnosis	NS
Lewnard *et al*[16], 2020	Gambia; Mali; Mozambique; Kenya; Bangladesh; India; Pakistan	Case-control study	2007-2011	Children aged 0–59 months with diarrhea	None	NS	Children without diarrhea	AGE caused by Salmonella, Shigella, Campylobacter, Aeromonas, and Vibrio spp Escherichia coli	NS	NS	NS	NS
Lewnard *et al*[17], 2020	Kenya, Bangladesh, India	Case–control study	2015-2019	Children aged < 5 years with acute respiratory infections and diarrhea in the 2 wk prior to the study	RV	PCV10/13	Children aged < 5 years unvaccinated for RV/PCV	Acute respiratory infection and diarrhea	NS	NS	NS	NS
At Thobari *et al*[15], 2020	Indonesia	Phase IIb randomized, double-blinded, controlled trial	January 2013 through July 2016	0-18 months of age	RV3-BB RV	NS	Placebo	NS	551 infants received ≥ 1 antibiotic in the 18-month observation period	956 antibiotic courses, 1.74 antibiotic uses per infant, mean duration of antibiotic use per child was 4.92 (± 1.86) d; no significant association between sex or vaccination group and the duration of antibiotic courses	NS	NS
Perez-Scha *et al* [14], 1990	Venezuela	Clinical/field trial	≥ 1 year and 1 year follow-up; sequential vaccine administration in	< 6 months of age	RV	NS	Placebo	The provided text does not mention a specific section on the history of	NS	NS	NS	NS

	4 periods: February-March 1985, June-July 1985, October 1985, and February 1986	infections (gastroenteritis) in the study

PCV: Pneumococcal conjugate vaccine; RV: Rotavirus vaccination/vaccine; NS: Not specified; AGE: Acute gastroenteritis.

Table 2 Risk of bias analysis for the included studies

Ref.	Selection				Comparability	Outcome			Total[1]
	Representativeness of exposed cohort	Selection of non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of the study	Comparability of cohorts based on basis of design or analysis	Assessments of outcomes	Was follow-up long enough for outcomes to occur	Adequacy of follow-up of cohorts	
Hall *et al*[7], 2022	1	0	1	1	1	1	0	0	5
Lewnard *et al* [16], 2020	1	0	1	1	2	1	1	1	8
Lewnard *et al* [17], 2020	1	0	1	1	1	1	1	1	7
At Thobari *et al*[15], 2020	1	1	1	1	2	1	1	1	9
Perez-Schae *et al*[14], 1990	1	1	1	1	2	1	1	1	9

[1]Quality score of < 3: Low quality of evidence; 3-6: Moderate quality of evidence; ≥ 7: High quality of evidence.

Conversely, Lewnard *et al*[16] investigated clinically attended, antibiotic-treated diarrhea across diverse age groups, identifying differential incidence rates among cohorts. However, this study did not explicitly delve into antibiotic switching or resistance. Notably, it encountered challenges in determining appropriate treatment for Shigella-associated diarrhea, highlighting the complexities in establishing suitable treatment strategies for certain causative agents. Lewnard *et al*[16] examined the impact of the pneumococcal conjugate vaccine (PCV) and rotavirus vaccines on illnesses treated with antibiotics in LMICs. Their study estimated a substantial annual prevention of millions of episodes, with potential additional prevention through universal vaccine coverage. Moreover, children who got at least three doses of PCV10/13 had lower chances of getting an acute respiratory infection that needed antibiotics. This was especially true for kids aged 24 months to 59 months , where the number of cases dropped by 19.7%[17].

Several studies, including the important randomized controlled trial by At Thobari *et al*[15], have highlighted the need for further investigation into antibiotic prescription practices in rotavirus-vaccinated populations. However, it is

important to note that while this trial provided essential insights into antibiotic usage patterns, it did not explicitly investigate antibiotic resistance development. In the early '90s, Perez-Schael *et al*[14] conducted a historical clinical trial in Venezuela that contributed contextually to the landscape of rotavirus vaccination. However, this study lacked detailed assessments of infections, antibiotic usage, or resistance patterns, limiting its direct relevance to the primary focus of our review.

The synthesis of these studies highlights the complexity inherent in unraveling the association between rotavirus vaccination and antibiotic resistance. Therefore, there is an urgent need for stronger, more standardized methods that include full analyses of antibiotic use, microbial causes, and resistance profiles in children who have rotavirus. Furthermore, the absence of consistent reporting standards across studies poses a significant challenge to synthesizing conclusive evidence. To address these limitations, the implementation of robustly designed, multi-centered prospective studies is essential. Such studies should encompass detailed antibiotic histories, resistance profiles, and clinical outcomes among rotavirus-vaccinated *vs* non-vaccinated cohorts. This approach could effectively bridge existing gaps and provide a clearer understanding of the intricate relationship between rotavirus vaccination and antibiotic resistance.

These collective findings underscore a significant association between rotavirus and pneumococcal vaccinations in children, resulting in a subsequent reduction in antibiotic prescriptions. This synthesis corroborates the pivotal role of vaccinations in public health, particularly in averting antibiotic-treated illnesses, contributing invaluable insights to global vaccination strategies. However, further research is imperative to harness these findings effectively to untangle the complexities of antibiotic resistance, establish refined treatment protocols, and discern broader implications for child health across diverse populations.

The reviewed studies strengths lie in their diverse geographical locations and comprehensive evaluations of vaccination effects on antibiotic utilization. Hall *et al*'s[7] longitudinal analysis and extrapolation to a national scale provides robust evidence supporting the association between rotavirus vaccination and reduced antibiotic prescriptions. Lewnard *et al*'s[17] investigation in LMICs adds a global perspective, highlighting the potential impact of vaccination coverage on reducing antibiotic-treated illnesses. However, limitations include variations in study designs, with some studies lacking explicit analysis of antibiotic resistance patterns and challenges in determining appropriate treatments for specific pathogens.

CONCLUSION

In conclusion, our systematic review provides evidence supporting the notion that rotavirus vaccination is associated with a reduction in the need for antibiotic prescriptions, which could potentially contribute to mitigating antibiotic resistance. The studies included in our analysis consistently showed a decrease in antibiotic prescriptions among children vaccinated against rotavirus, particularly after an AGE diagnosis. This finding suggests a potential role for rotavirus vaccination in mitigating antibiotic resistance. However, to fully understand the mechanisms underlying this association and to develop evidence-based strategies for combating antibiotic misuse and resistance, further research is necessary.

FOOTNOTES

Author contributions: Simhachalam Kutikuppala LV, Cozma MA, Maddineni G, Chorya HP, Tummala N, Godugu S, Chintala JS, and Găman MA have contributed to conceptualization, methodology, validation, formal analysis, investigation, writing original draft preparation, writing review and editing; Simhachalam Kutikuppala LV and Găman MA contributed to supervision; All authors have read and agreed to the published version of the manuscript.

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Country of origin: Romania

ORCID number: Lakshmi Venkata Simhachalam Kutikuppala 0000-0002-5685-6049; Matei-Alexandru Cozma 0000-0002-4998-0105; Mihnea-Alexandru Găman 0000-0001-7133-8875.

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