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Contents

Quarterly Volume 12 Number 5 December 25, 2023

REVIEW

MINIREVIEWS

ORIGINAL ARTICLE

Retrospective Cohort Study

Basic Study

World Journal of Virology

Quarterly Volume 12 Number 5 December 25, 2023

Contents

ABOUT COVER

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REVIEW

Emerging perspectives on RNA virus-mediated infections: from pathogenesis to therapeutic interventions

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Abstract

RNA viruses continue to pose significant threats to global public health, necessitating a profound understanding of their pathogenic mechanisms and the development of effective therapeutic interventions. This manuscript provides a comprehensive overview of emerging perspectives on RNA virus-mediated infections, spanning from the intricate intricacies of viral pathogenesis to the forefront of innovative therapeutic strategies. A critical exploration of antiviral drugs sets the stage, highlighting the diverse classes of compounds that target various stages of the viral life cycle, underscoring the ongoing efforts to combat viral infections. Central to this discussion is the exploration of RNA-based therapeutics, with a spotlight on messenger RNA (mRNA)-based approaches that have revolutionized the landscape of antiviral interventions. Furthermore, the manuscript delves into the intricate world of delivery systems, exploring innovative technologies designed to enhance the efficiency and safety of mRNA vaccines. By analyzing the challenges and advancements in delivery mechanisms, this review offers a roadmap for future research and development in this critical area. Beyond conventional infectious diseases, the document explores the expanding applications of mRNA vaccines, including their promising roles in cancer immunotherapy and personalized medicine approaches. This manuscript serves as a valuable resource for researchers, clinicians, and policymakers alike,

offering a nuanced perspective on RNA virus pathogenesis and the cutting-edge therapeutic interventions. By synthesizing the latest advancements and challenges, this review contributes significantly to the ongoing discourse in the field, driving the development of novel strategies to combat RNA virus-mediated infections effectively.

Key Words: RNA virus; Infections; Therapeutics; Drug target; Pathogenesis

Core Tip: This comprehensive review explores the intricate world of RNA viruses, highlighting innovative antiviral drugs, mRNA-based therapies, and advanced delivery systems. Our research delves into the nuances of viral pathogenesis and offers insights into combating infections. By synthesizing the latest advancements, this manuscript is a valuable resource for researchers, clinicians, and policymakers. We believe our work significantly contributes to the ongoing discourse and development of novel strategies against RNA virus-mediated infections.

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INTRODUCTION

Viruses are minute parasitic particles available in various shapes and sizes. These viral particles contain genetic material in the form of RNA or deoxyribonucleic acid (DNA), which can be single-stranded (ss) or double-stranded (ds), enclosed within a viral-encoded proteinaceous capsid coat. RNA viruses, characterized by exceptionally high genetic variability and phenotypic diversity, are intracellular obligatory parasites, capable of infecting a wide range of hosts[1,2]. They primarily target Eukarya and replicate using virally encoded RNA-dependent RNA polymerase (RdRp). In this type of viral replication, the synthesized RNA can serve as the genome, a copy of the genome, or messenger RNAs (mRNAs)[3]. Depending on the type of RNA acting as the genome, RNA viruses can be positive or plus strand or negative or minus strand.

RNA viruses are responsible for recurrent epidemics and occasional pandemics. Infections caused by respiratory and vector-borne RNA viruses, such as Influenza A virus, Zika virus, and West Nile virus, are of significant concern[4-6]. Other pathogenic RNA viruses affecting humans include Orthomyxoviruses, hepatitis C virus (HCV), Ebola virus, SARS, influenza, poliovirus, measles virus, and retroviruses such as adult human T-cell lymphotropic virus type 1 (HTLV-1) and human immunodeficiency virus (HIV). HIV, the causative agent of acquired immunodeficiency syndrome (AIDS), is a serious and prevalent viral disease. Currently, approximately 39 million people are living with HIV, and tens of millions have succumbed to AIDS since the beginning of the epidemic[7].

Recent outbreaks, such as the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2019, have underscored the importance of understanding the molecular mechanisms governing the evolution of RNA genomes and the potential for viral exposure[8]. Studies in this area have shed light on novel findings related to viral replication, host-virus interactions, and the development of antiviral therapies. Researchers continue to explore these aspects to develop strategies for combating RNA viral infections and mitigating their impact on global health.

RNA viruses are responsible for recurrent epidemics and occasional pandemics, causing significant global human morbidities and mortalities through virally induced emerging infectious diseases. The emergence and re-emergence of these viral infections have profound implications for public health, the overall economy, and the quality of life of affected populations. To mitigate their impact, it is essential to prevent the replication of RNA viruses, including retroviruses. Therefore, a comprehensive understanding of the replication and transcription processes of these pathogens is crucial[9].

Research on RNA viruses and the infections they cause serves as a pivotal foundation for vaccine development and the formulation of strategies for prevention, control, and corresponding therapeutic interventions. A deep comprehension of virus-host interactions is paramount to understanding the mechanisms governing viral replication and the associated pathological consequences. Advances in sequencing methods have been instrumental in revealing the significance of RNA-protein and RNA-RNA interactions during infections, providing valuable insights into the development of targeted therapies[10].

This review article aims to explore the current understanding of various RNA virus infections, focusing on their pathogenesis and the latest therapeutic interventions. Recent research in this field has led to the identification of novel drug targets, the development of antiviral agents, and the exploration of innovative vaccination strategies. Additionally, studies have elucidated the role of host immune responses and the viral factors contributing to disease severity, paving the way for personalized treatment approaches. Understanding the genetic diversity of RNA viruses, their evolutionary dynamics, and the mechanisms of viral transmission is essential for devising effective public health measures and preparedness strategies against future outbreaks. Moreover, ongoing research efforts continue to unravel the intricate interactions between RNA viruses and host cells, providing valuable information for the development of next-generation

antiviral therapies and vaccines.

RNA VIRUS REPLICATION AND TRANSMISSION

The viral DNA is protected and transported from cell to cell by very basic macromolecular structures known as envelopes or capsids in the extracellular environment**.** Only at the intracellular stage of their life viruses can produce distinctive compounds and engage in activities that are unique to living things. Creating a platform for genome replication and morphogenesis is one of these activities[11]. Due to the error-prone nature of RNA-dependent RNA polymerases of RNA viruses, they live as quasispecies with several variants within their populations[12]. A crucial phase in the life cycle of a virus is the replication of its genome. This procedure involves an intermediary complementary RNA strand for both plus- and minus-strand RNA viruses, but DNA for retroviruses[13].

The primary processes and entrance points for both enveloped and non-enveloped viruses are attachment to cell-surface receptors and transport of the viral genome to the host cell's cytoplasm. After attaching to receptors, which might be proteins, carbohydrates, or lipids, viruses enter the cell by one of two routes: endocytic or non-endocytic. After attaching to receptors, which might be proteins, carbohydrates, or lipids, viruses enter the cell by one of two routes: endocytic or non-endocytic[14]. Alphaviruses, Coronaviruses, Picornaviridae enteroviruses, and Flaviviruses are examples of positive-sense RNA (+RNA) viruses that significantly alter cellular membranes to act as platforms for replication and the assembly of new virions[15]. Since viral genomic RNA replication occurs in the cytosol of host cells, viruses must be able to distinguish between their own genome and many cellular RNAs that are present in cells, so that they amplify only their own genome. The co-optation of host RNA-binding proteins by RNA viruses to speed up replication or dodge host RNA breakdown mechanisms is expected[16].

All (+) RNA viruses can sequester host intracellular membranes to produce replication compartments (RCs). These RCs contain recruited host proteins and lipids as well as viral RNA and proteins, which together produce an environment that is favourable for RNA replication[17]. Capsids have not been found in RCs, suggesting that viral RNA is duplicated within RCs and then (+) RNAs are transferred outside to virion assembly sites. Figure 1 depicted the schematic representation of replication and transmission of positive-sense RNA viruses.

FACTORS CONTRIBUTING TO VIRAL ADAPTABILITY AND EVOLUTION

Similar to animal RNA viruses, plant RNA viruses may have evolved through three different pathways, including transfer of genes horizontally from hosts, parallel evolution with similar genetic components and coevolution or codivergence with hosts[18,19]. Three temporal phases of emergence were identified by Elena *et al*[20-22], these were: The host moves to a different species or the same species but in a different ecological condition, acclimatization to the new environment or host, and epidemiology in the recently arrived host population, often by adjusting to a new vector species or mechanism of transmission. The evolution and host adaptability of animal RNA viruses have piqued the interest of many researchers. The majority of animal RNA viruses have A-rich coding sequences, as reported by Kustin *et al*[23] They also proposed possible explanations such as codon usage bias, weakened RNA secondary structures, and selection for a particular composition of amino acids, concluding that host immunological forces may be the cause of similar biases in the makeup of coding sequences among animal RNA viruses.

ROLE OF HOST FACTORS IN VIRAL REPLICATION AND DISSEMINATION

Various strategies have evolved in (+) RNA viruses to utilize host cell resources. For replication to occur, the viral genomic RNA, along with viral and host components, must be actively attracted to the relevant subcellular membrane surfaces[13]. In the case of human poliovirus (PV), the host poly(rC)-binding protein 2 (PCBP2) plays a crucial role in recruiting RNA templates. PCBP2, an RNA-binding protein, facilitates cap-independent translation by binding to the internal ribosome entry site (IRES) in PV (+)RNA and stabilizing mRNA[20]. Upon binding to viral RNA, PCBP2 is cleaved by the PV-encoded RdRp precursor (3CD) or protease (3C) protein. Studies have demonstrated that the viral proteinase 3CD cleaves PCBP2, thereby suppressing viral translation[24]. Although cleaved, PCBP2 retains two RNA-binding sites, allowing it to attach to the cloverleaf structure at the PV (+) RNA's 5′ untranslated region (UTR). This interaction is vital for PV RNA replication as it brings together the 3′ and 5′ ends of the viral RNA through interaction with another host protein family, the poly(A)-binding proteins[25].

In the context of tomato mosaic virus (ToMV) replication, two Arabidopsis thaliana membrane proteins, TOM1 and TOM3, are essential. They interact with the helicase-like ToMV replication protein 130K, facilitating ToMV replication [26]. Additionally, the SNARE-like protein, human vesicle-associated membrane protein-associated protein A (VAPA), serves as a membrane anchor for HCV replication proteins[27]. HCV replication proteins NS5A and NS5B66 interact with VAPA, a contact crucial for the association of NS5A and NS5B with intracellular ER-derived membranes, which serve as the site of HCV replication[28]. Moreover, LSM1 protein aids in RNA recruitment in Brome mosaic virus and HCV. In PV, HCV, and coronaviruses, host heterogeneous nuclear ribonucleoproteins promote RNA recruitment and (+) RNA and (-) RNA synthesis[16,20].

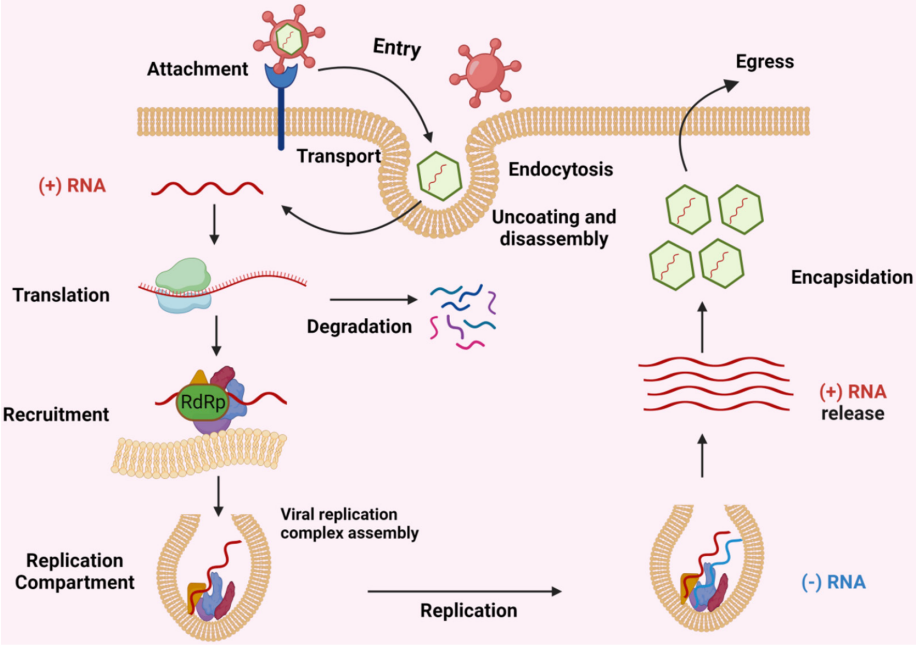

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Figure 1 Schematic representation of replication and transmission of positive-sense RNA viruses. RdRp: RNA-dependent RNA polymerase.

VIRAL-HOST INTERACTIONS AND IMMUNE RESPONSE

During a viral infection, the host's pattern recognition receptors (PRRs) detect viral pathogen-associated molecular patterns (PAMPs). These PAMPs represent distinct molecular attributes found in viruses that are absent in the host cell, enabling cells to differentiate between self and non-self entities, thereby initiating an immune response against infection [29,30]. The activation of these PRRs initiates intracellular signaling cascades involving adaptor proteins like MAVS and STING[6]. Subsequently, these adaptor proteins trigger the activation of kinases and transcription factors, which, in turn, promote the transcriptional upregulation of type I and type III interferons (IFNs) and the synthesis of antiviral proteins [31].

Efforts have been made to understand how virus infections affect host cell protein synthesis, but inhibiting host protein synthesis is not always necessary for successful virus replication[32]. Many viruses like paramyxoviruses, papovaviruses, and retroviruses typically do not block host protein synthesis during their replication. Moreover, mutant viruses that cannot effectively halt host protein synthesis are not necessarily impaired in their ability to replicate[13]. For instance, in the case of VSV mutants selected during persistent infections, they may have a reduced capacity to interfere with the host's translational processes, yet they can achieve higher virus titers during a lytic growth cycle compared to the wild-type virus[33,34]. In many instances, this inhibition is accompanied by an overall reduction in the rate of protein synthesis within the infected cell. This broad inhibition likely occurs at the initiation stage of protein synthesis, as observed by a decrease in the average size of polysomes in infected cells where examined[35].

Before the initiation of targeted cellular or humoral immune responses against a specific virus, the activation of apoptosis can serve as an initial defensive mechanism within host cells. This process aids in the removal of cells that have been infected by the virus, thereby restricting viral replication[36]. Molluscum contagiosum virus, equine herpesvirus 2, bovine herpesvirus 4, human herpesvirus 8, and herpesvirus saimiri encode FLICE-inhibitory proteins (FLIPs) that exhibit structural similarities to FLICE (caspase-8)[37]. These FLIPs engage with the Fas-associated death domain protein adapter within the host cell[20].

Studies on candidate genes and genome-wide associations have yielded important information on the genetic foundations of many infectious illnesses. The loss-of-function mutation known as CCR5Δ32, which results in the absence of CCR5 expression on the surface of host cells, confers resistance to HIV infection in homozygous individuals[31]. Polymorphic variations in HLA genes have been associated with a wide range of infectious diseases, including RNA viruses such as SARS, influenza, HIV, hepatitis C, rabies, West Nile fever, rubella, mumps, and measles, among others. Genetic association studies of this nature are pivotal for pinpointing HLA alleles that might be correlated with immune responses offering protection. Variations in HLA alleles have prompted inquiries into their potential role in the distinct immune responses observed between mild and severe coronavirus disease 2019 (COVID-19) cases, such as delayed immunoglobulin M (IgM) responses and elevated S protein immunoglobulin G titers in non-intensive care unit patients [38,39].

ANTIVIRAL DRUGS

RNA virus-mediated infections include HCV, SARS, influenza, Ebola, polio, measles, HIV, HTLV-1, Respiratory syncytial virus (RSV), and others. Most RNA viruses have single-stranded or double-stranded RNA as their genetic material. Viruses primarily possess RNA-dependent RNA polymerase for genome replication or have reverse transcriptase for genome replication. Idoxuridine was the first antiviral drug approved in 1963. Since then, 90 antiviral drugs categorized into 13 functional groups have been approved for the treatment of 9 infectious diseases. Antiviral drugs approved for RNA virus-mediated infections include trifluridine, vidarabine, entecavir, zidovudine, didanosine, lamivudine, abacavir, nevirapine, efavirenz, rilpivirine, ritonavir, indinavir, lopinavir, simeprevir, paritaprevir, raltegravir, elvitegravir, palivizumab, tenofovir disoproxil fumarate, sofosbuvir with ribavirin, amantadine, zanamivir, rimantadine, laninamivir octanoate, and favipiravir[40].

In the realm of influenza treatment, antiviral drugs primarily include adamantanes (M2 ion channel blockers) which can block an ion channel formed of M2 protein encoded by the M gene in influenza A virus. This category comprises two classes of drugs: Amantadine and rimantadine. Additionally, neuraminidase inhibitors, approved for use against both influenza A and influenza B viruses, include major drugs such as zanamivir, oseltamivir, Peramivir, and Laninamivir [41]. Another class, the RNA-Dependent RNA Polymerase Inhibitors, includes favipiravir, which hampers viral RNA synthesis. Notably, the absence of favipiravir-resistant viruses is a remarkable property, indicating its effectiveness as an antiviral medication. A novel class, the Polymerase Acidic Endonuclease Inhibitor, encompasses baloxavir marboxil, which inhibits the cap-dependent endonuclease of the viral RdRp complex. This disruption restricts mRNA production and prevents subsequent viral protein synthesis. Baloxavir marboxil is one of the most recently developed anti-influenza drugs[42].

Challenges in developing anti-influenza drugs stem from the virus's antigenic evolution mechanisms: Antigenic shifts and drifts in surface glycoproteins. Immunization against these processes is challenging due to their natural immunity combat mechanisms. Major limitations in antiviral treatment include antiviral resistance of most human influenza A virus strains to M2 inhibitors and the need for in vivo disease models for influenza-related research. Evaluating antiviral treatment efficacy poses challenges from the standpoint of drug resistance. One of the main hurdles in treating influenza is the emergence of drug-resistant influenza viruses due to current antiviral regimens. Alternative treatments include drug combination therapies that synergistically minimize drug resistance and reduce drug toxicity. Commonly used antiviral combination therapies include Oseltamivir + zanamivir (targeting the same viral protein), Baloxavir + favipiravir (targeting Cap-Dependent Endonuclease & RNA-Dependent RNA Polymerase), Baloxavir + oseltamivir (targeting Cap-Dependent Endonuclease & Neuraminidase), and Oseltamivir + amantadine + ribavirin (Triple-combination antiviral drug treatment targeting M2 Ion Channel, Neuraminidase & RNA-Dependent RNA Polymerase) [43]. Additionally, next-generation influenza virus inhibitor candidates in early-stage development include EIDD-2801 and Pimodivir, a cyclohexyl carboxylic acid analogue targeting the polymerase PB2 subunit to hinder influenza virus replication[44].

Moving to HCV treatment, initial antiviral efforts utilized IFNα monotherapy followed by ribavirin (RBV), a synthetic triazole guanosine analogue active against both DNA and RNA viruses. Pegylated IFN, a modified interferon with a prolonged pharmacokinetic profile, showed favorable results. Subsequently, direct-acting antivirals (DAAs) emerged to directly interfere with viral proteins, marking a significant breakthrough. Protease inhibitors (PIs) like boceprevir and telaprevir were among the first DAAs, substantially increasing sustained virologic response (SVR) rates. However, these drugs accelerated the generation of resistance-associated substitutions, leading to virological breakthroughs in almost all treated individuals. Compared to the prior standard regimen of Peg-IFN–RBV, Peg-IFN–RBV–triple therapy raised SVR rates in treatment-naive patients by about 30%[45].

The COVID-19 pandemic spurred progress in antiviral medication development, introducing both DAAs and host-based antivirals. Commonly used DAAs include remdesivir and molnupiravir, inhibiting RNA replication through interaction with RdRp. Nirmatrelvir targets the main protease or 3-chymotrypsin-like protease, while favipiravir and ritonavir are used for mild to moderate COVID-19 cases[46,47]. However, these drugs have limitations. Molnupiravir, in particular, has mutagenic potential for both the virus and the host. Host-based antiviral drugs like camostat and ivermectin target transmembrane protease serine 2. Other options include fluvoxamine, thapsigargin, and plitidepsin[48].

For HIV, various antiretroviral drugs have been developed, including Non-nucleoside reverse transcriptase inhibitors, Nucleoside reverse transcriptase inhibitors, PIs, Fusion inhibitors, CCR5 antagonists, Integrase strand transfer inhibitors, and Post-attachment inhibitors. Combination antiretroviral therapy or highly active antiretroviral therapy (HAART) uses these drugs in combination. Food and Drug Administration (FDA)-approved HAART treatments include bictegravir, emtricitabine, and tenofovir alafenamide, cabotegravir, and rilpivirine, doravirine, lamivudine, and tenofovir disoproxil fumarate, among others. Direct-Acting Anti-HIV Agents such as cabotegravir, doravirine, and islatravir, along with host-based antivirals like the monoclonal antibody ibalizumab, are being used or are in the developmental stage[47,49]. Despite their efficacy, these drugs pose various risks and side effects, including elevated liver enzyme levels, gastrointestinal toxicity, rashes, benign hyperbilirubinemia, nausea, headache, anemia, leukopenia, reversible peripheral neuropathy, lactic acid elevation, low phosphate levels, and CNS toxicity[50].

In the fight against polio, large-scale oral vaccination programs have been implemented through the Global Polio Eradication Initiative. However, no specific antipicornavirus drugs have been FDA approved. Promising candidates include pirodavir (capsid binders), rupintrivir (protease inhibitors), Enviroxime (protein 3A inhibitors), ribavirin (nucleoside analogues), and compounds like MDL-860, discovered as a broad-spectrum inhibitor of viruses, although its exact mechanism of action remains unknown[51,52].

Ebola virus, a highly lethal pathogen, lacks FDA-approved drugs or vaccines. However, several potential drugs are under investigation, including favipiravir, amiodarone, amodiaquine, chloroquine, clomiphene, toremifene, Brincidofovir, sertraline, and BCX4430[53].

RSV is a negative-sense, ssRNA virus that primarily causes acute lower respiratory tract infections in infants, children, adults, and immunocompromised individuals. Currently, the FDA has approved two drugs for RSV treatment: ribavirin (a guanosine analogue) and palivizumab (a monoclonal antibody)[54]. Several antiviral candidates against RSV are under clinical research and trial stages, including REGN2222, MEDI8897, and Motavizumab. Additionally, various fusion inhibitors, nucleoprotein inhibitors, nucleoside analogues, and non-nucleoside inhibitors are under development for RSV therapy[54].

HTLV-1 is an RNA virus mainly responsible for HTLV-1-associated diseases such as Adult T-cell leukemia (ATLL) and neurological disorders like HTLV-1 Associated Myelopathy (HAM). It is a retrovirus that affects CD4+ cells and, to some extent, CD8+ cells and dendritic cells. The treatment approach for ATLL diseases mainly involves a combination of drugs such as interferon α and zidovudine (IFN-α/AZT). However, there is no established treatment approach for HAM disorder[55]. These are some of the most commonly encountered RNA virus infections and the commonly used antiviral agents/drugs for their treatment.

RNA-BASED THERAPEUTICS

RNA holds significant potential for therapeutic applications. Growing understanding and recent advancements in RNA studies have paved the way for various innovative RNA-based therapeutic approaches. Several RNA-based therapeutic methods are gaining popularity and receiving clinical approval for use. These approaches offer certain advantages over antiviral drugs, conventional protein targeting, and DNA-based medicines. The key advantage lies in targeting the RNA of the virus, providing a broader and more efficient target. Small molecular drugs, in contrast, target about 0.05% of the human genome. Moreover, many targets of disease lack clearly defined active regions for binding of small molecule.

RNA-based treatments face significant challenges in terms of intracellular trafficking and metabolic stability. However, researchers have explored a variety of strategies to overcome these obstacles[56].

TYPES OF RNA-BASED THERAPEUTICS

RNA interference

RNA interference (RNAi) is an *in-vivo* cellular process which leads to silencing of RNA expression by using ds RNAs, this provides an intrinsic defensive mechanism against invading viruses and transposable elements. miRNAs and siRNAs are small oligonucleotide sequences of 20-22 nucleotides with definite structures composed of 5′-phosphate and 3′-hydroxyl endings and two 3′-overhang ribonucleotides on each duplex strand. Within the RNA-induced silencing complex, the endoribonuclease Dicer isolates the guide and passenger strands by cutting dsRNAs. While the passenger siRNA strand is broken down by the argonaute2 (AGO2) protein, the guide siRNA strand attaches itself directly to the target RNA and initiates AGO2-mediated cleavage[57]. When siRNAs bind to the promoter regions, they can also cause chromatin remodelling and histone changes in the nucleus, which silences transcription in addition to destroying cytoplasmic RNAs. This therapeutic approach has a lot of potential to be use in HIV, Influenza, SARS-CoV treatment[58].

Antisense oligonucleotides

In antisense oligonucleotides (ASOs)-mediated gene regulation, short single-stranded oligonucleotides (12-24 nt) are utilized. These oligonucleotides are complementary to specific RNA sequences through Watson-Crick base pairing, enabling them to alter the expression of proteins, reduce, or restore their expression[59]. There are two types of mechanisms used to modify expression: One is Occupancy-Mediated Degradation: In this mechanism, ASOs induce target mRNA cleavage by RNase H1 or ribozymes. The ASOs lead to the degradation of the target RNA, and 2nd is Occupancy-Only Mechanism in this mechanism, the target RNA is not directly degraded. Instead, various mechanisms are employed to modify expression. These include altering RNA splicing using splice switching, blocking miRNA binding to the target RNA, inhibiting or activating translation, and triggering nonsense-mediated mRNA decay[60].

CRISPR-based genome editing

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a prokaryotic defense system widely applied in genome editing. The CRISPR-associated protein (Cas) system enables precise genome sequence editing in mammalian cells and organisms, leading to the target gene's irreversible knockout or knockin. This system relies on guide RNA and Cas nucleases. The complex recognizes the protospacer adjacent motif sequence in the target RNA, initiating its activity. For effective genome editing, the Cas nuclease cleaves either the double-stranded DNA or a single-stranded RNA at the designated spot[61]. The most commonly used Cas systems are Cas9 and Cas13. The Cas9 system can target both double-stranded DNA and single-stranded RNA. This technique finds applications in detecting SARS-CoV-2, with CRISPR-Cas13-based assay designs used for detecting 67 diseases, including SARS-CoV-2, Zika virus, and dengue fever, among others. The CRISPR/Cas9 system aids in studying the regulation pathway of the influenza virus, representing an emerging field in developing antiviral therapies against diseases like HIV, Hepatitis, and SARS[62].

Aptamer: It is synthetically designed chemical antibodies, are single-stranded oligonucleotide sequences that specifically bind to and inhibit protein expressions. Aptamer selection is based on the methodical evolution of ligands by exponential enrichment. Aptamers function primarily by interfering with interactions between disease-related targets, such as those between proteins or between receptors and ligands[63]. Aptamers can also deliver therapeutic agents to specific cells. They have applications in controlling SAR-CoV-2 infection; slow off-rate modified aptamers (SOMAmers) are DNA-based aptamers that bind to specific S protein fragments of SARS-CoV-2, preventing virus interaction with ACE-2 receptors[64]. Aptamers like anti-CCR5 are designed to prevent the interaction of HIV with the T-cell GPCR receptor. They serve as prospective anti-HIV/AIDS drugs, offering targeted delivery of various therapeutic options through CCR5-targeted aptamers and aptamer-siRNA conjugates[65]. Aptamers are also used in viral disease diagnosis; aptasensors, electrochemical diagnostic tools, demonstrate advantages such as low cost, specificity and early detection for influenza A and HA glycoprotein virus particles[66].

mRNA-based therapeutics

mRNA is generally single-stranded and is transcribed from the antisense strand of DNA, carrying information about the expression of functional proteins. mRNA-based therapeutics represent the future of treating various refractory diseases, including infectious diseases, metabolic genetic disorders, cancer, cardiovascular diseases, and others. In this therapy, exogenous mRNA is introduced with the help of a carrier, acting as a vaccine or therapeutic agent that expresses the necessary functional protein. This approach offers several advantages over conventional therapies, including higher efficiency, faster design and production, adaptability, and lower costs. These benefits are possible due to a planned manufacturing method developed for in vitro transcribed mRNA[67].

However, there are challenges associated with mRNA-based therapies. The anionic nature of cell membranes makes it difficult for mRNA to translate functional proteins in the cytoplasm. Additionally, mRNA has a median intracellular half-life of approximately 7 hours, and efficient carriers are crucial for overcoming cellular barriers, improving immunogenicity, and addressing stability issues. Moreover, mRNA may trigger immunological reactions and related toxicities, hindering the development of mRNA-based treatments[68,69].

The basic steps for designing and manufacturing mRNA-based therapeutic agents include mRNA design and synthesis, mRNA entrapment, pharmacodynamics and safety evaluation, manufacturing, and clinical trials[70]. Quality control measures are essential, such as codon optimization for mRNA encoding the antigen, ensuring mRNA sequence identity and integrity, assessing nucleic acid quantity, 5′ capping, poly A tail length, optimizing 5′-UTRs and 3′-UTRs, and ensuring mRNA purity. During the drug delivery process, mass spectrometry analysis, nuclear magnetic resonance analysis, evaluation of lipid electric charges and ratios, assessment of lipid impurities, and transfection efficiency are crucial. For mRNA-lipid nanoparticle drugs, encapsulation efficiency, particle size, storage conditions, and zeta potential must be carefully considered[67,70].

Apart from the conventional linear mRNA form, there are other structural forms such as self-amplifying RNA derived from alphaviruses, circular RNAs, noncoding RNAs, and competitive endogenous RNAs. These diverse forms of mRNA can be utilized for therapeutics[71,72]. Correct delivery of mRNA inside living systems is pivotal, and various delivery systems like lipid nanoparticles (LNP), polymeric nanoparticles, cationic nanoemulsions, protamine-condensed mRNA, exosomes, extracellular vesicles, and mesoporous silica are used[73]. These delivery systems utilize electrostatic interactions, hydrogen bonds, or coordination interactions through methods like thin-film hydration, nanoprecipitation, or microfluidic mixing. Nanoparticle-based delivery systems enhance cell uptake, facilitate lysosomal escape, and accelerate translation, maximizing mRNA availability. Achieving effective in vivo distribution of mRNA necessitates tissue-targeted delivery of mRNA-based therapies[74]. Precision nanoparticle engineering has been developed to cross biological barriers, expanding its applications in various therapeutic areas for mRNA-based drug delivery[75].

LNPs are a popular mRNA-based delivery method targeting the liver. Current research focuses on improving LNP platforms for administration to additional tissues. Through the exact and predictable customization of LNPs to transport mRNA, Cas9 mRNA/single-guide RNA, and Cas9 ribonucleoprotein complexes to target organs *via* intravenous injection into the liver and lungs, selective organ targeting has emerged as a therapeutic method. Another critical aspect of drug delivery is the route of administration. While the majority of disorders can be treated with intravenous administration specific administration routes are tailored to the targeted organ or organ system[76,77].

Over the past three to four years, mRNA-based therapeutics have gained significant popularity due to their role in designing treatments for COVID-19 and are now extensively explored for their applications in other viral infections, specifically RNA virus infections such as influenza, SARS, HIV, HCV, RSV, and various cancer therapies. A growing number of well-funded biotechnology companies, including Moderna, CureVac, BioNTech, Argos Therapeutics, RaNA, Translate Bio, Ethris, Arcturus, and Acuitas, are investing billions of dollars in mRNA therapy. Clearly, one of the most compelling topics in medication research is mRNA, which is worth investigating in the long run[67].

MICRORNA (MIRNA) PATTERN AND ITS ROLE IN GLIOMAS

Circulating microRNAs, have gained significant attention in the field of cancer research, as potential non-invasive diagnostic biomarkers for various types of tumors, including gliomas (a type of brain tumor)[78]. miRNAs can be found in different body fluids, including blood (serum and plasma), and cerebrospinal fluid and can be transported between cells, including tumor cells and neighboring normal cells, through exosomes so these can be used as reliable biomarkers [79]. miRNAs interfere the protein translation through complementary base-pairing, or degrade mRNA, thus dysregu-

lation of miRNAs lead to tumor progression[80]. The miR-21 has the potential to predict the radiation necrosis compared to tumor progression[78]. The potential utility of miR-128 and miR-342-3p as biomarkers for assessing glioma grades and monitoring treatment response has been advocated[81]. Indeed, the profiling of these miRNAs can provide valuable insights into the presence, type, and stage of the tumour. Consequently, early detection and accurate diagnosis are crucial for patient prognosis and treatment planning, and survival.

For rapid and early detection of miRNAs high sensitive methods such as a toehold-mediated strand displacement reaction, the enzyme-free surface plasmon resonance imaging biosensing method, and the ultrasensitive electrochemical method, should be integrated with existing diagnostic modalities, such as imaging and molecular profiling (genetic and epigenetic markers), to enhance diagnostic accuracy and guide treatment decisions. But challenges are the heterogeneity nature of gliomas, the varying level of sensitivity and specificity of miRNA, small number of studies, lack of standardized protocols for miRNA isolation, quantification that makes miRNA to accurately detect all glioma types and stages[82]. With the advancement of glioma biology and miRNA function, possibility of miRNA-based tests may eventually become a valuable part of the diagnostic and screening toolkit for glioma patients.

DEVELOPMENT AND APPLICATION OF MRNA-BASED VACCINATION

Vaccination plays a important role in dealing with communicable diseases, remarkably contributing to global public health. In simple terms, vaccination aims to generate immunity against specific diseases by using vaccines. Conventional vaccine candidates mainly include whole organism vaccines (live attenuated or inactivated pathogens), subunit vaccines, viral vectors, *etc*., which have been crucial in disease prevention. However, the scalability, speed of development, and ability to respond to newly emerging pathogens of these conventional vaccination platforms are often limited.

Recently, mRNA-based vaccination has emerged as the most advanced technology offering various benefits. It provides a flexible framework for the quick and focused development of vaccines against infectious illnesses, such as viral outbreaks and new infections. mRNA vaccines also have the advantage of being developed and produced more quickly than traditional vaccinations, which frequently need expensive and time-consuming manufacturing procedures. When it comes to responding to emerging infections or developing variants, mRNA vaccines offer unparalleled flexibility and speed because they can be designed and manufactured in a few of weeks[83].

During recent times, mRNA-based vaccination against SARS-CoV-2 has been a groundbreaking discovery in tackling diseases. The design of mRNA vaccines is adaptable to various diseases by simply changing the mRNA sequence encoding the required antigen. However, their efficient delivery poses a substantial challenge due to their susceptibility to degradation, poor stability, and obstacles in reaching the targeted areas of action[84].

The delivery of mRNA-based vaccines is a major challenge due to the internal environment inside the cytoplasm and the need to pass through the cell membrane. Various delivery systems have been developed, among which nanoparticles have emerged as promising tools in mRNA vaccine delivery, overcoming the inherent limitations of naked mRNA molecules. These nanoscale delivery and protection systems offer effective cellular absorption, defense against enzymatic breakdown and controlled mRNA payload release. Furthermore, nanoparticles can be developed to increase balance, extend their duration of circulation, and enable targeted administration to immune cells or organs, enhancing the immunogenicity and effectiveness of mRNA vaccines[85].

Various types of nanoparticle-based carrier systems include LNPs, polymeric nanoparticles, peptides, and protamine-based delivery systems, as well as cationic nanoparticles. Among them, for the administration of mRNA vaccines, LNPs have become the most popular class of nanoparticles. Due to their hydrophobic core, LNPs can enclose and safeguard mRNA, facilitating effective cellular absorption and intracellular release. This strategy is highly effective in designing various mRNA vaccines against infectious diseases, including COVID-19[86,87]. LNPs are positioned as prospective tools for successful vaccination techniques due to their outstanding safety profiles, high transfection efficiency, and capacity to elicit robust immune responses. This nanoparticle-based delivery system for mRNA is also useful in addressing other issues like cancer immunotherapy, personalized medicine, and therapeutic interventions for genetic disorders[88].

TYPES AND MECHANISM OF ACTION OF MRNA VACCINES

Self-amplifying mRNA, trans-amplifying mRNA, and conventional mRNA are the three forms of mRNA vaccines that are now on the market. Conventional mRNA vaccines, also known as non-replicating or non-amplifying mRNA vaccines, mainly consist of untranslated regions (5′UTR, 3′UTR) and the coding part of mRNA, which by transcription produces one copy of the immunogenic protein[71]. Self-amplifying mRNA vaccines are genetically modified mRNA, incorporating engineered replicons from self-replicating RNA viruses. They possess 5′ and 3′ conserved sequence elements (CSE) that regulate viral RNA synthesis and facilitate attachment to viral or cellular proteins. Self-amplifying RNA contains non-structural proteins 1-4 (nsP 1-4) sequences[89]. Trans-amplifying mRNA is also genetically modified mRNA with 5′ and 3′ CSE. Trans-amplifying mRNA requires two RNA genes to be co-delivered: the mRNA without nsP 1-4 and the mRNA encoding nsP1-4 genes[90].

In mRNA vaccination technology, mRNA is synthesized outside the body, injected, and then transported across cell membranes for translation in the cytoplasm. Once in the cytoplasm, the mRNA is translated into the necessary protein by ribosomes. In the case of naturally occurring mRNA, this process occurs after the mRNA moves from the nucleus or cell membrane. The poly-A tail get attached to the poly-A-binding protein during translation, and the eIFs attach to the 5′UTR cap to start translation[70]. Ribosomes convert each codon, consisting of three nucleotides in the translated portion of the

mRNA, into an amino acid. After injection, immune cells internalize mRNA-LNPs, leading to the release of mRNA from the LNPs. Ribosomes recognize the mRNA, translating it into antigenic proteins. These proteins are broken down and processed by proteasomes, resulting in small peptides presented on the cell surface by major histocompatibility complex class I (MHC I) molecules, and activates CD8+ T lymphocytes to eliminate infected cells. These produced antigen can be broken down further by lysosomes, loading small peptides on major histocompatibility complex class II (MHC II) molecules, recognized by CD4+ T lymphocytes. These cells stimulate B cells to stimulate humoral immune responses and inflammatory cytokine release to stimulate cellular immunological responses. Successful mRNA translation into the necessary antigen requires recognition of the 5′ cap, poly-A tail, 5′UTR, 3′UTR, and translated region of the synthesized mRNA vaccine by the ribosomes[67].

Optimizing mRNA vaccines is essential for enhancing their stability, safety, and efficiency. Modifications in 5′ cap of the 5′UTR and also in the poly(A) tail of 3′UTR can regulate translational efficiency of mRNA. For instance, converting the mRNA cap into phosphorothioate when the mRNA vaccine is transfected in immature dendritic cells may increase stability and expression. Furthermore, by including altered nucleosides into the mRNA, the Toll-like receptor activation may be decreased or eliminated, improving the vaccine's safety *via* nucleoside modification[91].

DELIVERY SYSTEMS

The most commonly used mRNA vaccine delivery systems include LNPs, Polymeric nanoparticles, peptides, protein nanoparticles, protamine nanoparticles, and other systems like cationic lipid amphiphiles. Because they may effectively transfer mRNA intracellularly by merging with the lipid bilayer of early endosomes, lipid-based nanoparticles are frequently preferred. Through this procedure, the mRNA is shielded from RNase breakdown during systemic circulation and is allowed to enter the cytoplasm. These LNPs primarily has three important components, *i.e.*, 40%-50% ionizable lipids, 38%–45% cholesterol, and 10%–12% helper phospholipid. In certain cases, a fourth component, such as 1%-2% PEGylated lipid, is added. LNPs have been utilized for delivering mRNA vaccines and drugs against diseases like COVID-19 and influenza[92,93].

Polymeric nanoparticles involve the addition of low molecular weight polyethyleneimine with polyethylene glycol, which is then linked to cyclodextrin. Conjugation with cyclodextrin has been proven as a reliable and safe method for delivering mRNA. This approach has versatile applications and may lead to the development of specific antibodies[94].

Peptides and protein-based nanoparticles are extensively used due to their excellent biocompatibility and accessibility. Amphipathic peptides, with cationic or amphipathic amine groups (arginine), can facilitate delivery of mRNA into the cells. These peptides binds electrostatically to the mRNA, forming nano-complexes[95].

Protamine nanoparticles leverage the net positive charge of protamine, a cationic protein primarily composed of positively charged amino acids. Protamine can complex with nucleic acids, such as RNAs, enhancing their stability and shielding them from enzymatic degradation by nucleases. This property facilitates their delivery to specific tissues[96]. Protamine's cationic nature, attributed to an arginine-rich sequence, enables it to interact with negatively charged mRNA, making it valuable in the design of mRNA-based vaccines.

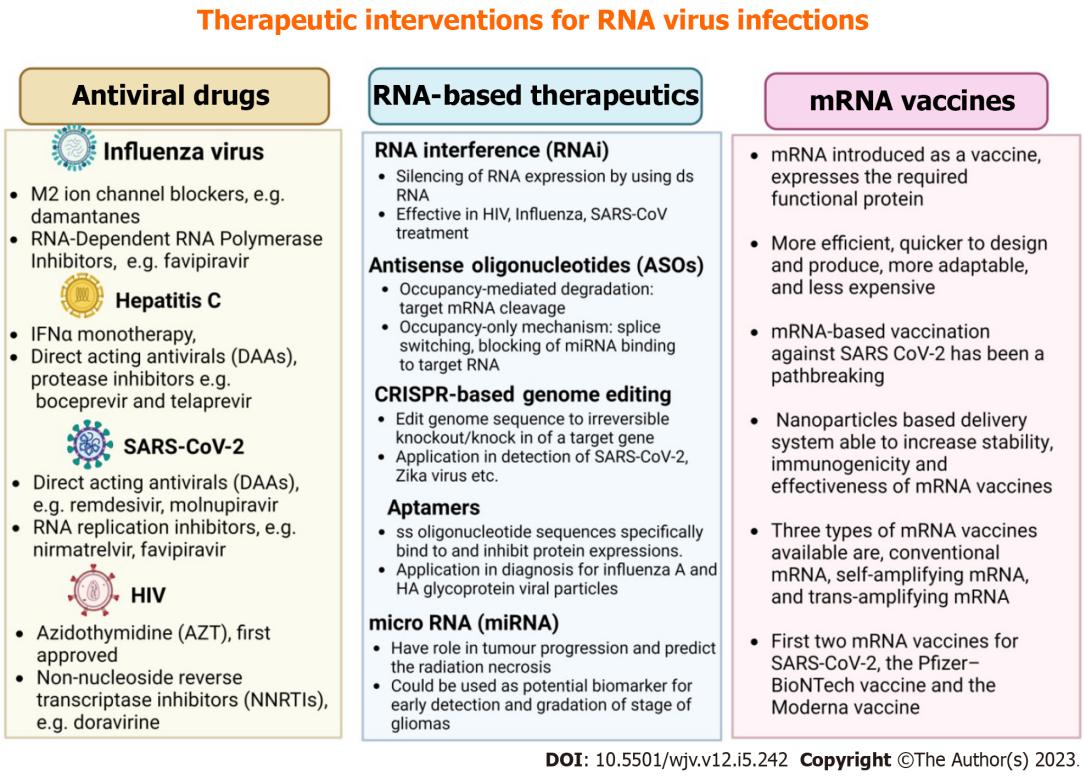
APPLICATION OF MRNA VACCINES

mRNA vaccines are primarily designed to generate immunity against infectious diseases and cancers. Due to the COVID-19 pandemic, these vaccines were developed more quickly, and as a result, the FDA approved the first two mRNA vaccines for SARS-CoV-2: The Moderna vaccine (mRNA-1273) and the Pfizer-BioNTech vaccine (BNT162b2)[97]. In addition to SARS virus mRNA vaccines, various mRNA vaccines for different viral infections such as influenza, HIV, Zika, and Rabies are under development. Numerous potential mRNA vaccine candidates are undergoing clinical trials, including mRNA-1345 against RSV (in phases two and three trials developed by Moderna), mRNA-1273 against SARS-CoV-2 variant B.1.351 (in phase two trials), and mRNA-1893 against Zika virus (in phase two trials). Apart from their application in preventing infectious diseases, mRNA vaccines also have broad applications in cancer treatment[98,99]. Figure 2 summarizes therapeutic interventions like antiviral drugs, RNA-based therapeutics, and mRNA vaccines for RNA virus infections.

FUTURE PERSPECTIVES AND CHALLENGES

The idea of personalized medicine may bring a great revolution in the field of medicine. Personalized medicine, an emerging practice of medicine uses a person's genetic profile to guide decisions which are made in regards to the prevention, diagnosis, and treatment of the disease[100]. The use of personalized medicine may be beneficial in many aspects, such as diagnostic accuracy improvement, better disease prevention, targeted therapy, reducing side effects, and the health care cost and promotion of research[101].

For instance, despite of rapid advancement in medical science, the modern medicine could not provide adequate treatment for COVID-19. In case of COVID-19 pandemic, it was established that observing the genetic background of each patient can contribute greatly to the drug effectiveness[102]. After the arrival of the challenges regarding treatment of coronavirus infection, the crucial roles of personalized medicine were realized by the physicians and healthcare workers.



DOI: 10.5501/wjv.v12.i5.242

Figure 2 Summary of therapeutic interventions for RNA virus infections has been summarized. HIV: Human immunodeficiency virus; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; IFN: Interferon.

Hence, utilization of personalized medicine may stay as a potential therapeutic strategy for RNA virus mediated infections.

Previously, in late 2003, a coronavirus, *i.e.*, SARS-CoV-1 caused an outbreak of severe acute respiratory illness (now called SARS), and acute respiratory distress syndrome type findings[103]. In 2012, another coronavirus witnessed to cause Middle East respiratory syndrome, having a case-fatality rate of more than 35%. An outbreak of a disastrous pandemic occurred in late 2019, which in due course spread all over the world. Out of all these three coronavirus outbreaks the SARS-CoV-2 infection was newer and the first two outbreaks helped us to prepare for this third one[104].

The absence of suitable animal models, an insufficient understanding of the correlates of immune protection, and limited pharmaceutical industry investment are obstacles that affect the development of an HIV vaccine[105].

If the viral pathogens are zoonotic, it must be needed a prevention barrier to reduce their chances of first introduction to human population. 'One health' approach reduces the prevalence of viral pathogens with high zoonotic potential in animals and which in turn reduces the viral introduction into human population[106]. It is quite easy to monitor, treat or vaccinate the domestic animals, but is complicated for the wild animals. An inclusive surveillance plan must be executed for the wild animals in order to recognize the pathogens having possibility of transmission to human[107]. Advanced sequencing methods can be used for the surveillance to find out the variations which could have enhanced the zoonotic potential or pathogenicity[108]. Surveillance of bush meat market may be important for detecting such zoonotic viral pathogens.

For the management of any future pandemics, we must be ready with some preventable approaches. These approaches may be the non-pharmacological approach (such as mask wearing and social distancing), vaccine anticipation, and anticipating therapies to reduce morbidity and mortality[107].

Orthomyxoviruses, HCV, Ebola illness, SARS, influenza, polio, measles, and retroviruses including adult HTLV-1 and HIV are among the human diseases caused by RNA viruses. The genetic material of RNA viruses is RNA, which can be single-stranded or double-stranded[109]. Reverse transcriptase produces viral DNA that can be integrated into host DNA through its integrase activity, and viruses can also use RNA-dependent RNA polymerases to make copies of their genomes. Retroviruses, on the other hand, have two copies of their single-strand RNA genomes. Due to the error-prone nature of RNA-dependent RNA polymerases of RNA viruses, they live as quasispecies with several variants within their populations[110].

CONCLUSION

In this review article, we have highlighted the pathogenesis and recent advances in the treatment of RNA virus-mediated infections. We discussed RNAi and various RNA-based antiviral drugs, as well as the development of RNA vaccines and the challenges associated with their administration. Different types of vaccines exhibit distinct efficacy, and we

emphasized various strategies to enhance vaccine effectiveness. Additionally, our focus was on host-directed therapies, which represent an antiviral strategy. However, the development of these therapies poses significant challenges. Overcoming these challenges is crucial to transforming host-directed therapies into potent antiviral treatments.

FOOTNOTES

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MINIREVIEWS

Routine pediatric vaccinations during the COVID-19 pandemic: A review of the global impact

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic has put standard, routine childhood vaccinations at risk worldwide. The disruption in vaccine coverage has resulted in a negative impact on the health of children, with some races, ethnicities, age groups, areas of settlement, and parts of the world affected more than others. This literature review studied and examined the impact of COVID-19 on infant, child, and adolescent vaccinations. Retrospectively, the analysis showed a decline, delays, or interruptions in the coverage of vaccines during the pandemic and a decline in some countries' pre-pandemic and post-pandemic eras. Necessary attempts and efforts should be made for these delayed and missed vaccinations, as failure to do so could put children's health at risk. Thus, priority should be directed at instituting catch-up programs to support vaccine uptake and decrease the probability of acquiring vaccine-preventable diseases.

Key Words: Pediatric; Vaccination; Coronavirus disease 2019; Humans; Pandemics

Core Tip: Studies worldwide have reported a decline in vaccination rates among the pediatric population because of the coronavirus disease 2019 pandemic. The disruption in vaccine coverage has resulted in a negative impact on the health of children, with some races, ethnicities, age groups, areas of settlement, and parts of the world affected more than others. Government efforts should be directed towards reversing these missed vaccinations.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic profoundly impacted the healthcare systems worldwide, resulting in significant and unintended consequences such as the disruption of routine health services, including immunization. A range of factors have contributed to these disruptions, such as travel restrictions, policies designed to reduce person-to-person contact, and concerns about viral exposure, resulting in the cancellation or postponement of patient visits. One result of these disruptions is a significant reduction in vaccine coverage, particularly in regions such as north Africa and the Middle East, south Asia, Latin America, and the Caribbean[1,2]. In the United States, data shows a decline in vaccination rates during the pandemic period, particularly during age-limited preventive care, and although vaccination rates rebounded during the expanded primary care period, they have yet to reach pre-pandemic levels[3]. To address this issue, China's Centers for Disease Control and Prevention (CDC) developed successful catch-up vaccination guidelines for children who missed or experienced delays in vaccination due to COVID-19[4]. Although sub-Saharan Africa experienced the lowest disruptions, data reveals a significant decline in vaccine coverage globally against vaccine-preventable diseases such as measles, diphtheria, tetanus, and whooping cough. Moreover, there are growing concerns on the heightened risk of outbreaks of other vaccine-preventable diseases such as polio[1]. Understanding the early impacts of the pandemic on vaccine coverage will help immunization programs determine how to continue to serve the health care needs of the population. Thus, this review aims to explore the impact of the COVID-19 pandemic on global immunization for other communicable diseases.

Methodology

A mini-review was carried out to describe the epidemiological elements of the present global immunization decline in the pediatric population during the COVID-19 pandemic. An electronic literature review was conducted primarily using Google Scholar, MedLine Plus, and PubMed. The search for the assembled data was not limited to peer-reviewed studies published between December 2019 and September 2023. Grey literature sources were also visited to learn more about the decline in routine pediatric vaccinations during the pandemic. When selecting publications or manuscripts, keywords such as COVID-19 pandemic, immunizations of children, vaccinations, and disruption of vaccines were considered.

COVID-19 pandemic and routine childhood vaccination rates in the United States

Prior to the pandemic, the United States ranked highest coverage of most recommended pediatric vaccines due to its vaccination guidelines for documentation of vaccines for daycares and schools[3]. In September 2020, routine infant, child, and adolescent weekly vaccinations, showed that it was lower than 2019. Vaccination coverage had major distinctions between race and ethnicity across all ages and periods. The lowest was in African American children, and although disparities were present pre-pandemic, COVID-19 made these differences more significant, especially in the 18-mo-old age group[3]. When comparing the age groups and race for up-to-date vaccinations, the African American pediatric population was low on the spectrum. In the age group of 7 mo, the highest vaccine receiving population was in Asian infants (88.0%) and the lowest in African American infants (61.0%); in the 6-year-old age group, the highest was among Hispanic children (79.0%), and lowest was in African American children (70.0%); in the adolescent age group the lowest vaccinated were among African American adolescents (51.0%) and Caucasian adolescents (51.0%)[3]. Another disparity was seen in children living in rural areas, having increased missed vaccination doses, compared to their peers living in urban areas (33.3% *vs*. 15.2% unvaccinated children)[5]. These findings make it imperative to ensure that all children are targeted for their required vaccinations no matter their living situation.

Figure 1 compared the average weekly vaccine doses in 2019 and 2020 among those < 24 mo, 4-6 years, 11-13 years, and 16-18 years in the United States. Before the COVID-19 pandemic months: 2019 = 01/06/19-03/16/19; 2020 = 01/05/20-03/14/20; age-limited preventative care months: 2019 = 03/17/19-05/18/19; 2020 = 03/15/20-05/16/20; expanded primary care months: 2019 = 05/19/19-10/05/19; 2020 = 05/17/20-10/03/20. The vaccines administered in the study were: Children < 24 mo-hepatitis B (HepB); rotavirus; diphtheria, tetanus, pertussis (DTaP); *Haemophilus influenzae* type B (Hib) conjugate; measles, mumps, rubella (MMR); inactivated polio; varicella-zoster; and 13-valent pneumococcal conjugate. Children 4-6 years-MMR, varicella-zoster, and inactivated polio. Children 11-13 years-human papillomavirus (HPV), Tetanus toxoid, reduced diphtheria toxoid, acellular pertussis vaccine, and quadrivalent meningococcal conjugate. Adolescents 16-18 years-HPV and quadrivalent meningococcal conjugate[3].

The weekly vaccination rates shown in Figure 1, were measured by age group, period, and year. The pre-pandemic period in 2020 across all age groups was comparable to that in 2019. The period that saw a decline across all age groups was the age-limited preventative care in 2020 compared to 2019. The expanded primary care, was the only time that showed increased vaccination rates amongst all age groups. These findings further demonstrated how the COVID-19 pandemic affected the pediatric population regarding immunizations and how much this vulnerable population recovered from diseases.

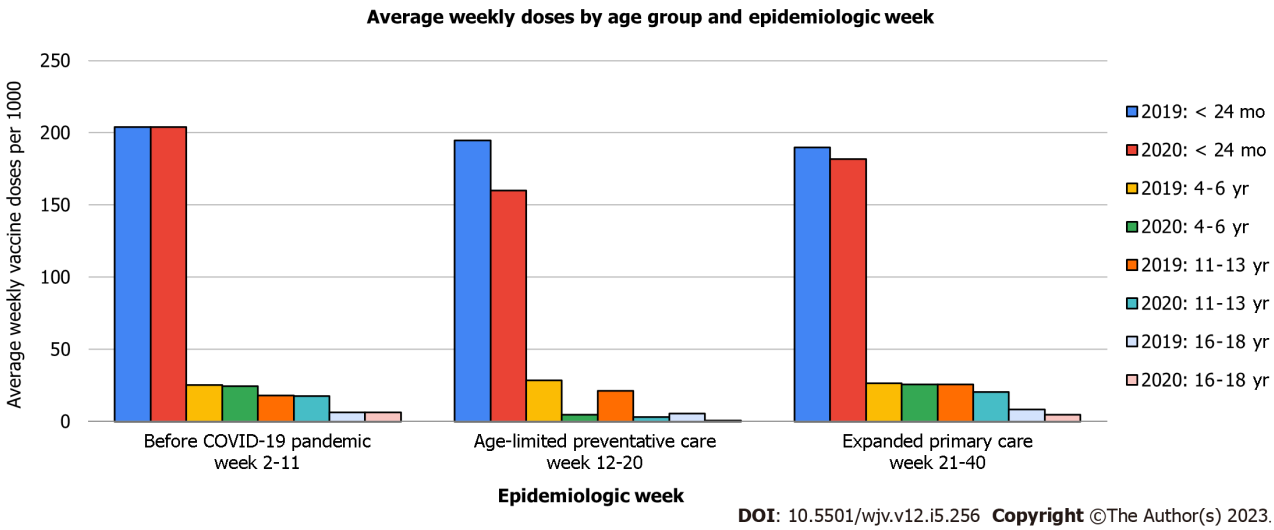

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Figure 1 Average weekly vaccine doses by age groups and epidemiologic week[3].

Figures 2 and 3 compared the monthly vaccine doses administered in vaccination clinics in the United States from January 2020 to June 2020 with the baseline doses administered in December 2019[4]. Vaccinations compared are Bacillus Calmette-Guerin (BCG) vaccine, DTaP vaccine, diphtheria and tetanus toxoid vaccine, hepatitis A vaccine, HepB vaccine, Japanese encephalitis vaccine, measles-containing vaccine (MCV) vaccine, group A meningococcal polysaccharide vaccine, group A and C meningococcal polysaccharide vaccine, polio vaccine, and total vaccines. As depicted in Figure 2, weekly vaccination doses declined from the baseline in December 2019, especially in February 2020; however, most vaccines took a positive step in the catch-up phase of delivery doses in the following six months. One possible reason for the increase in required vaccinations administered to the pediatric population, beginning in late March 2020, could be the strategies executed to promote childhood vaccinations and reaching out to patients that were past due by the vaccine safety datalink, a collaboration with the CDC[6].

Discussion

The usual immunization schedule was significantly impacted by the COVID-19 pandemic, particularly in the pediatric population. Even after taking COVID-19 precautions and returning to regular activities, the gaps in vaccine coverage raise the risk of vaccine-preventable infections. According to studies, the disruptions in normal immunizations caused by the pandemic provided risk of a 10.0% increase in mortality from diseases that can be prevented by vaccination[2]. Diphtheria-tetanus-pertussis, third dose vaccine (DTP3), and MCV first dose (MCV1) coverage were estimated to have fallen by more than 7.0% worldwide compared to expected coverage in the absence of COVID-19. More than 8 million additional children missed the DTP3 and MCV1 beyond expected estimates of vaccination gaps for 2020[2]. The global vaccination rate against tuberculosis (BCG vaccine), poliomyelitis vaccine (polio 3), and HepB third dose (HepB3) also dropped in 2020[7,8]. Over half of the African countries recorded a reduction of vaccination rates with Tanzania (polio 3) and Djibouti (DTP, Hep3, 3 doses of Hib vaccine (Hib3), 3 doses of pneumococcus conjugate vaccine (PCV3), MCV1, and MCV2) mainly impacted. In Asia, the COVID-19 pandemic caused a decline in pediatric vaccination coverage for almost all vaccinations. Larger decline (7.0%) of the BCG vaccination was observed in India. DTP3 has the highest reduction in Nepal (9.0%). The most pronounced decline observed for MCV1 occurred in Indonesia (12.0%) while the largest reduction in polio 3 vaccination was recorded in the Democratic People's Republic of Korea. Significant reductions in Europe occurred in Bulgaria, Ukraine, and Montenegro with MCV1, MCV2, BCG, and HepB3. Reductions in routine vaccination rates also occurred in the Americas with BCG, MCV1, PCV3, and polio3. In Canada, report of the Childhood National Immunization Coverage Survey collected in 2021, during the COVID-19 pandemic, showed that in-school vaccination programs for adolescents experienced delays and interruptions which differed across jurisdictions; however, by March 2021, national vaccination coverage rates were similar to the pre-pandemic level[9]. In Oceania, reductions were noted in some countries, notably in Samoa for MCV1 and Kiribati for MCV2[7,8]. Although the first half of COVID-19 was the most severe and caused the biggest impact, the second half showed promise with the increased number of vaccinations being administered; but despite this increase, millions of doses were still not being delivered. Some regions have been on the rise in their attempts to recover from the impact of COVID-19, but there are some areas that are still under the average for vaccinations in the pediatric population[2]. Regions such as sub-Saharan Africa were already below the global target before the pandemic, therefore recovery would still leave the pediatric population vulnerable to preventable diseases and affecting the long-term health of the children in that area[2]. Addressing the pre-pandemic gaps in coverage of missed childhood vaccinations needs to be considered as well as ensuring that all children receive their necessary immunizations; whether it was missed pre-pandemic or post-pandemic[5].

Moreover, those over six months of age are recommended to get the COVID-19 vaccine, according to the CDC, for the best defense[10]; despite that, COVID-19 typically causes minor illness, low hospitalizations, and infrequent post-acute consequences in young children[11,12]. However, there has been a correlate with COVID-19 infection in children and adolescents developing new-onset type 1 diabetes (T1D) mellitus and potentially other post-acute sequelae[13].

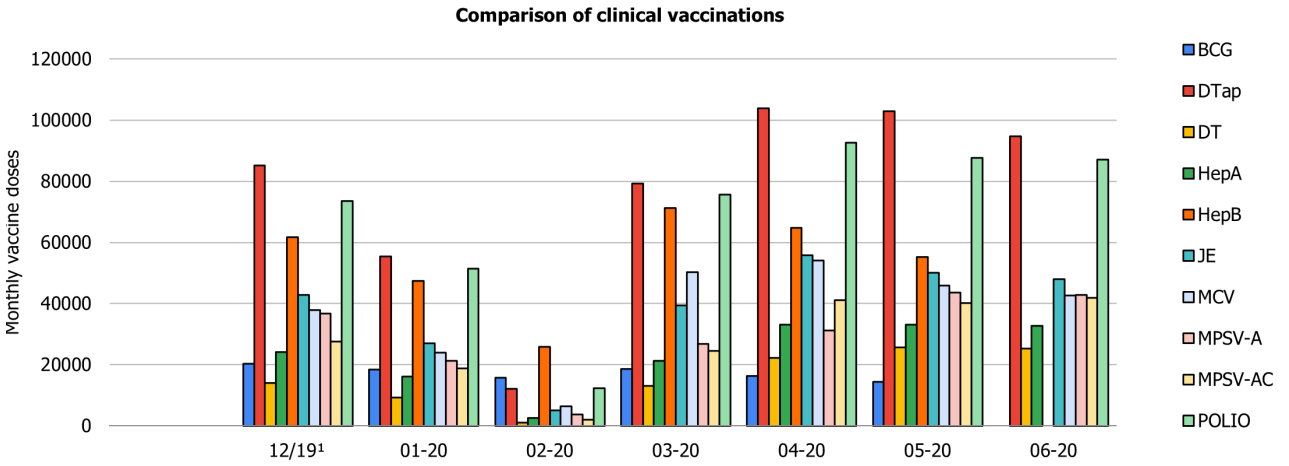

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Figure 2 Monthly vaccine doses of Bacillus Calmette-Guerin, diphtheria, tetanus, pertussis, diphtheria and tetanus toxoid, hepatitis A, hepatitis B, Japanese encephalitis, measles-containing, group A meningococcal polysaccharide, Group A and C meningococcal polysaccharide, and poliovirus vaccinations from December 2019 to June 2020[4]. [1]Marks the baseline when vaccine doses were administered in December 2019. BCG: Bacillus Calmette-Guerin; DTaP: Diphtheria, tetanus, pertussis; DT: Diphtheria and tetanus toxoid; HepA: Hepatitis A; HepB: Hepatitis B; JE: Japanese encephalitis; MCV: Measles-containing; MPSV-A: Group A meningococcal polysaccharide; MPSV-AC: Group A and C meningococcal polysaccharide; Polio: Poliovirus.

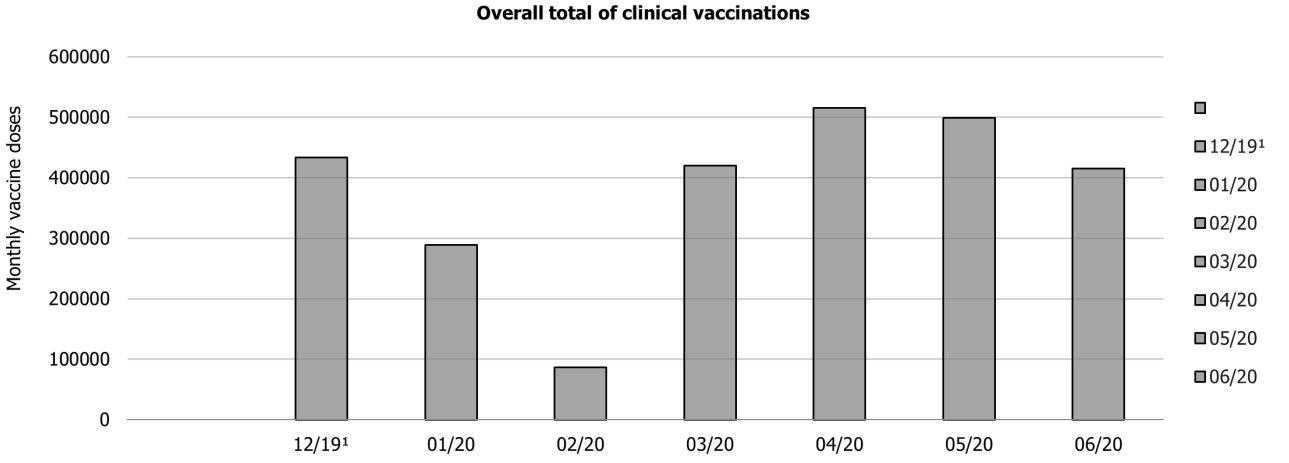

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Figure 3 Overall total vaccinations from December 2019 to June 2020[4]. [1]Marks the baseline when vaccine doses were administered in December 2019. Bacillus Calmette-Guerin vaccine doses administered were not available in June 2020. Hepatitis B vaccine doses administered were not available in June 2020.

Compared to pre-COVID-19 pandemic levels, the median glucose and hemoglobin A1C levels in newly diagnosed T1D children increased by 6.43% and 6.42%, respectively[14]. The pandemic of COVID-19 has increased the risk of juvenile new-onset T1D, diabetic ketoacidosis (DKA), and severe DKA worldwide[14]. During the early period of the B.1.1.529 (omicron) variant's predominance in the United States, the rate of COVID-19-associated hospitalization among children under five years of age peaked at 14.5 per 100000 in January 2022[11]. Roughly, this was five times the rate during the period of predominance of the B.1.617.2 (delta) variant in 2021[11]. It is worthy of note that 63.0% of infants and kids hospitalized in 2022 due to COVID-19 did not have any underlying medical issues[11]. It is of importance to encourage national pediatric associations to create national plans for integrating the COVID-19 vaccine into current immunization schedules, and according to the European Academy of Paediatrics campaign, "Vaccinate your child" should have support from national pediatric associations to reinstate postponed routine immunizations or vaccinate missed children[15].

Communities need to have a more inclusive system to provide opportunities for all children, no matter their living situation in order to receive their vaccinations. In 2019, it was found that coverage for the MCV1 vaccine which covers the first dose for measles was lower among children living in remote rural areas, compared to children living in urban areas [5]. Even a year after the COVID-19 pandemic started, challenges and gaps in the vaccines and immunizations system continued. Vaccinations systems are limited in tracking outside age groups with the current delay by notifying who and what vaccination is required for that given child. More strategic programming needs to be in place to reach the pediatric

population who missed vaccinations. Regions around the world can begin to develop stronger and healthier communities with a more up-to-date immunization requirement system, but if vaccines are still delayed, COVID-19 will continue to impact the children's health now and the future generations.

CONCLUSION

Studies worldwide have reported a decline in vaccination rates among the pediatric population because of the COVID-19 pandemic. Government efforts should be directed toward reversing these missed vaccinations. Strategies need to be strictly followed during times of crisis to prioritize the maintenance and reduced delays in routine childhood vaccination programs. Initiatives include low-income countries obtaining their missed vaccines through an organized programs such as increasing vaccine availability in remote areas, decreasing the wait times to care, as well as increasing public health awareness. To address these vaccine deficiencies and to maintain children's health protection from preventable communicable illnesses like polio and measles, public health procedures must be strictly followed. These infections can have negative consequences, including mortality, for younger children. Thus, stringent protocols should be safeguarded to avoid the increase in risk of other fatal illnesses, and emphasize the importance of providing consistency in maintaining childhood vaccinations to ensure the health and protection of preventable diseases in children.

FOOTNOTES

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MINIREVIEWS

Hepatitis E infection: A review

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Abstract

Hepatitis E virus (HEV) is a small non-enveloped virus that is transmitted *via* the fecal-oral route. It is a highly common cause of acute hepatitis, particularly in low to middle income regions of Asia, Africa, and Central America. Most cases are self-limited, and symptomatic patients usually present with acute icteric hepatitis. A subset of patients including pregnant women, older men, those with pre-existing liver disease and immunocompromised patients however, may develop severe disease and hepatic failure. Immunocompromised patients are also at risk for chronic infection, and their immunosuppression should be decreased in order to facilitate viral clearance. HEV can also present with a variety of extra-intestinal manifestations including neurological, renal, hematological, and pancreatic derangements. The gold standard of diagnosis is HEV ribonucleic acid detection *via* nucleic acid amplification testing. Currently, there are no approved treatments for Hepatitis E, though ribavirin is the most commonly used agent to reduce viral load. Studies assessing the safety and efficacy of other antiviral agents for HEV are currently underway. HEV vaccination has been approved in China, and is currently being investigated in other regions as well. This review article aims to discuss the epidemiology, pathogenesis, presentation, diagnosis, complications, and treatment of Hepatitis E infection.

Key Words: Hepatitis E; Acute hepatitis; Chronic hepatitis; Viral hepatitis; Vaccination

Core Tip: Hepatitis E is a common viral infection that has been increasing in developed nations. It usually causes a self-resolving acute hepatitis. It can sometimes lead to chronic hepatitis, and even cirrhosis/hepatic failure. Several subtypes exist, however the types responsible for infections in humans are generally spread *via* pork consumption or contaminated water. Treatment is usually supportive, however, ribavirin has shown efficacy in those with severe or chronic infection. Immunocompromised and pregnant patients should be evaluated with particular caution. Vaccination is currently licensed in China, and many studies are underway assessing vaccination efficacy in other nations as well.

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INTRODUCTION

Hepatitis E virus (HEV) is a small non-enveloped virus in the *Hepeviridae* family. It was first discovered in the 1980s in a military camp in Afghanistan and was identified *via* electron microscopy in an individual who had symptoms of acute viral hepatitis[1]. Globally, HEV accounts for a significant proportion of liver disease, and is responsible for up to 70% of adult sporadic hepatitis cases in endemic regions. It is thought to be the most common etiology of acute viral hepatitis with an estimated incidence of 20 million cases yearly[2]. HEV is primarily transmitted *via* the fecal-oral route, and is responsible for multiple epidemics in developing countries within Asia and Africa[2]. However, it has become increasingly prevalent as a zoonotic viral infection in developed countries as well. Though HEV infection is self-limited in many cases, mortality rates and the incidence of fulminant hepatic failure (FHF) are significant in older male patients (6.5-10% mortality), pregnant patients (25%-30% mortality), and those with chronic liver disease (22%-43% mortality)[3]. Management is usually supportive, however, immunocompromised patients with chronic infection as well as high-risk populations may require antiviral treatment in order to prevent progression of liver disease and associated morbidity and mortality. HEV vaccination is currently approved in China, and multiple randomized control trials are underway in other endemic regions including Pakistan and Bangladesh.

EPIDEMIOLOGY

Hepatitis E is a hepatic infection caused by the HEV, a positive sense ribonucleic (RNA) virus, and is considered a global health issue. According to the World Health Organization, an estimated 20 million cases of HEV infection occur yearly resulting in 70000 deaths[4]. Particularly endemic to developing countries, HEV can be found in Asia, Africa, and Central America, and is especially prevalent in low to middle income regions of those areas[5]. The primary route in endemic areas of infection is fecal-oral, making areas with poor water sanitation particularly susceptible[6]. Four genotypes (1-4) are largely implicated in cases of HEV infection. In the above mentioned endemic regions, genotype 1 and 2 are predominantly the causative strains[5]. Sporadic cases and outbreaks can also occur, both in developed and under-developed regions, for which genotypes 3-4 are largely responsible and are most often secondary to zoonotic transmission, primarily from domestic pigs and wild boars[7]. Additionally, contaminated water can lead to viral transmission through shellfish, fruit, and salads[8,9]. In the United States (US), HEV is largely considered a travel-associated disease, usually brought into the country by travelers returning from endemic areas. However, a retrospective study of nationwide hospitalizations from 2010-2017 found that the incidence of HEV in the US has increased nearly two-fold[10]. In autochthonous (locally acquired) cases, HEV is thought to be predominantly caused by zoonotic transmission, usually originating from undercooked pork[4,7].

PATHOGENESIS

HEV is primarily spread fecal-orally *via* contaminated water or food (*e.g.* undercooked pork). The virus is a single-stranded, positive-sense RNA virus and is divided into two genera: Piscihepevirus and Orthohepevirus, the latter of which is divided further into 4 species (A-D). Interestingly, HEV-C is primarily spread by rats, and only shares 50%-60% identity with HEV-A. Some case reports describe HEV-C infection in transplant recipients, however, its infectious potential in humans remains unclear[11]. HEV-A has seven genotypes, of which 1, 2, 3, 4 and 7 infect humans[12]. HEV primarily targets hepatocytes however, until recently, the route of HEV reaching the hepatocytes was poorly understood. It is now thought that the virus first replicates enterically, with studies finding HEV RNA and ORF2 antigens in intestinal crypts of chronically infected patients. From here, the virus is thought to then enter the portal circulation and infect hepatocytes causing inflammation. The mechanism of viral entry into the hepatocyte is still poorly understood[13]. After entering the hepatocyte, the HEV genome is released into the cytoplasm where the virus hijacks intracellular machinery to replicate vital proteins and the RNA genome. ORF4 is critical for the replication process, and ORF3 is necessary for viral release from infected cells[12]. The HEV virion is shown in Figures 1 and 2.

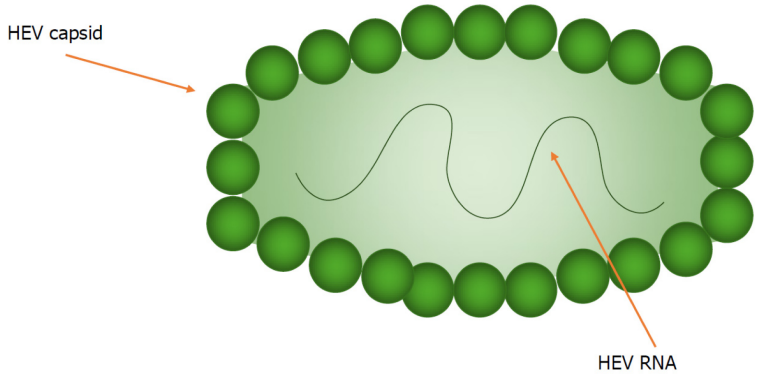

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Figure 1 Naked hepatitis E virus virion structure. HEV: Hepatitis E virus.

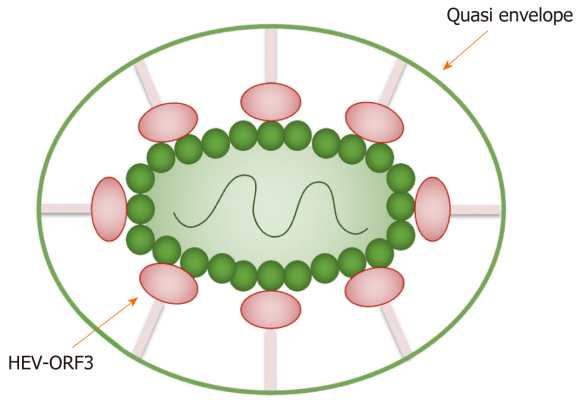

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Figure 2 Quasi-enveloped hepatitis E virus virion structure. HEV: Hepatitis E virus.

The humoral immune response in conjunction with cellular immunity limits viral replication and allows the host to clear the infection, which is largely responsible for the self-limited nature of majority of cases[14]. In acutely infected patients, anti-HEV immunoglobulin M (IgM) antibodies peak in 6 wk, followed by anti-HEV IgG antibodies for long-term (years to decades) protection[13]. Acute infections are also associated with elevated T cells, with increases seen in both CD4+ and CD8+ populations, and subsequent release of pro and anti-inflammatory cytokines interferon (IFN) gamma and interleukin (IL)-10[15]. Further immune protection is provided by the innate lymphoid cell response, with natural killer (NK) cells combating viral infection with both cell-mediated cytotoxicity and by producing IFN gamma[12]. The same immune response responsible for limiting HEV infection is also largely the cause of hepatocellular damage and liver inflammation[12]. In their efforts to clear HEV from the host, CD8+ and NK cells along with the production of interferons, cause intrinsic damage to hepatocytes, leading to hepatitis[16].

CLINICAL PRESENTATION

Hepatitis E infection can present with a wide range of clinical manifestations. Most commonly, infected hosts remain asymptomatic. Symptomatic cases present as acute icteric hepatitis, which occurs in 5%-30% of infected hosts. This presentation includes a prodromal phase that lasts up to one week, manifested as fever, nausea, vomiting, and malaise [17]. Following the prodromal phase, dark urine and jaundice signal the onset of the icteric phase. During this time, mortality rates range from 0.5%-4%, however symptoms usually resolve spontaneously within a week[18]. More severe presentations can occur, such as fulminant HEV infection and/or progression to chronic HEV infection (sustained HEV replication for more than 3 mo). The populations most susceptible to these outcomes are pregnant and immunocompromised patients, such as solid organ transplant recipients and those with human immunodeficiency virus (HIV). Extra-hepatic complications can occur in both acute and chronic HEV infection, ranging from renal impairment to neurological symptoms (see section on complications). Extra-hepatic manifestations seem to be driven both directly by HEV replication and indirectly by immune system mediated effects[19].

DIAGNOSIS

Diagnosis of acute HEV infection initially involves the detection of anti-HEV antibodies (IgM). IgM antibodies appear in the acute phase of infection, and are detectable approximately 4 d after onset of jaundice. They may remain detectable for up to 5 mo. Anti-HEV IgG antibodies develop shortly after IgM, and remain in the serum for up to 14 years post-infection [20]. Sensitivity of traditional immunoassays range from 90-97%, with false positives up to 2.5%[21]. Immunocompromised patients may have delayed or absent seroconversion to anti-HEV antibodies, rendering this diagnostic modality insufficient in which case nucleic acid amplification testing (NAT) to detect HEV RNA from stool, serum, or liver biopsy can be used[21].

The gold standard test for confirming acute HEV hepatitis is detection of HEV RNA *via* NAT from serum, stool or on liver biopsy. However, there are several factors that make RNA detection a faulty method. Firstly, detection of HEV RNA is dependent on time of patient presentation. Following onset of illness, RNA is detectable in the serum up to 4 wk later, and up to 6 wk in the stool. Secondly, viral load can remain low and therefore even during the detectable periods, may not be captured by NAT[20]. The availability of HEV RNA testing in commercial labs remains limited, further restricting its use. Furthermore, the methodology of HEV NAT has not been standardized and large variability in sensitivities has been noted in the various techniques. Greater sensitivity has been noted in real-time reverse transcription polymerase chain reaction (RT-PCR) compared to nested RT-PCR assays[22]. A flowchart of HEV diagnosis is outlined in Figure 3.

COMPLICATIONS/SPECIAL POPULATIONS

Hepatic complications

Chronic HEV infection is primarily seen in immunocompromised hosts, and is exceedingly rare in immunocompetent patients. These patients largely consist of solid organ transplant patients, however other cohorts, such as HIV patients and chemotherapy patients, have also been described. These patients largely remain asymptomatic, and usually only have mild to moderate derangement in liver enzymes[23]. HEV-induced liver cirrhosis is a complication only seen in immunocompromised patients, often seen in HIV patients with low CD4 (< 200) counts or recent organ transplantation. Patients who have undergone solid organ transplant and are infected with HEV have a 50% chance of progressing to liver cirrhosis over several years, with 10% of patients reaching that point within 5 years[23-25].

Patients with pre-existing liver disease are at increased risk for severe HEV infection and liver failure, and should be evaluated with caution. A recent meta-analysis of 18 studies by Qiu *et al*[26] found a 35.8% rate of liver failure and 14.3% mortality rate in patients with chronic liver disease and superimposed HEV infection. Patients with cirrhosis had a two-fold increase in risk of liver failure and four-fold increase in risk of death compared to patients without cirrhosis. Similarly, a retrospective study by Tseng *et al*[27] demonstrated that HEV infection increases the rate of liver disease progression in patients with chronic hepatitis B (HBV) infection. The study also found an increased risk of mortality in patients in HBV-cirrhosis compared to non-cirrhotic patients (30% *vs* 0%, $P < 0.001$). Other studies have shown similar results regarding the effects of HEV superinfection in patients with pre-existing liver disease, prompting a discussion on vaccination for HEV in all patients with chronic liver disease in endemic regions (see section on vaccination)[28-30].

Pregnancy

HEV infection in pregnancy can be life-threatening with mortality rate up to 30% with HEV genotype 1 infection largely due to the development of HEV-induced FHF[12]. Studies have shown that pregnant patients with a progesterone receptor gene mutation, PROGINS, had reduced NK cell activity along with altered humoral and cellular immune responses[31]. Other studies have shown that pregnant patients have higher levels of tumor necrosis factor alpha, IL-6, and IFN-gamma, and that these cytokines had a significant positive correlation with HEV viral load, serum bilirubin, and prothrombin time. This raises the possibility that increased severity of HEV infection in pregnant patients may be mediated by increased levels of cytokines in the serum[12,32]. Non-host complications of HEV infection in pregnancy include vertical transmission of the disease, increased preterm births, stillbirths and neonatal mortality[33].

Extrahepatic manifestations

HEV infection can be complicated by extrahepatic manifestations ranging from neurological to renal complications. These manifestations can occur in both acute and chronic infection and are thought to arise from a combination of HEV replication in involved tissues and immune system related effects. Neurological pathologies have been widely reported and are seen largely in HEV genotypes 1 and 3. Reported disorders included Guillain-Barre syndrome, Bell's palsy, polyradiculopathy, neuralgic amyotrophy, acute transverse myelitis, and acute meningoencephalitis[18]. The pathophysiology behind neurological symptoms in HEV infection remains unclear, however it is hypothesized that the host immune response plays a large role, with studies showing that immunocompetent patients are more likely to have neurological complications than immunocompromised ones[18].

Renal injury is seen in both acute and chronic HEV infection, again with HEV 1 and 3 genotypes. Renal biopsies in affected patients show histological patterns of membranoproliferative glomerulonephritis and membranous glomerulonephritis. These complications are seen in both immunocompetent and immunocompromised patients. The pathophysiology is poorly understood, though it is possible that immune complex deposition, such as that seen in hepatitis C infection, could be the cause[18,34].

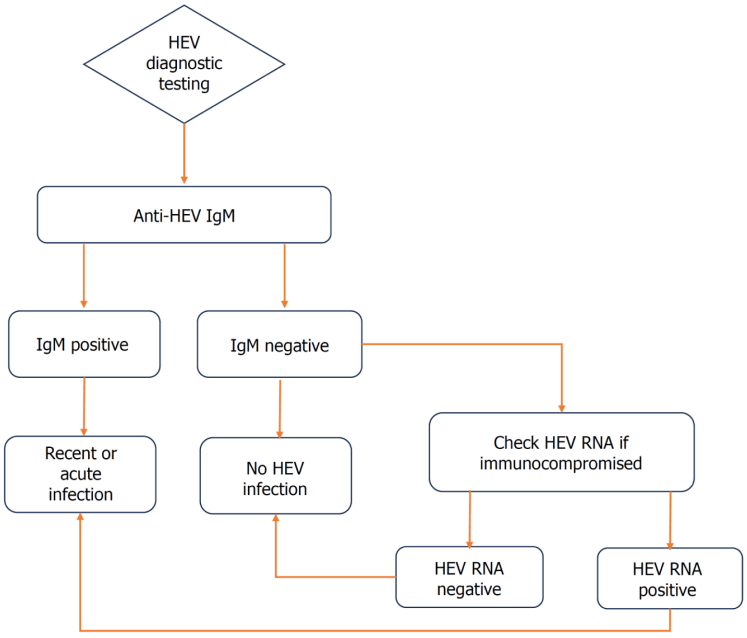

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Figure 3 Flowchart of diagnostic testing for acute hepatitis E virus infection. HEV: Hepatitis E virus; IgM: Immunoglobulin M.

Hematological complications such as aplastic anemia and thrombocytopenia have been reported, though the mechanism behind these complications is not well understood. Monoclonal gammopathy of unknown significance (MGUS), was found to have the prevalence of 26.2% according to a study by Woolson *et al*[35]. Studies have yet to determine whether the inflammatory state of HEV leads to increased prevalence of MGUS or if it is immunosuppression caused by MGUS that predisposes to HEV infection. Brown *et al*[36] found a significantly elevated risk of MGUS and multiple myeloma with infectious hepatitis (RR 1.82; 95%CI 1.25-2.65), though the study did not identify specific etiologies of the infectious hepatitis. Severe thrombocytopenia (platelet count < 20000) has been reported in rare cases of HEV infection, and as of 2010 there were only 6 known reported cases. Though the cause remains unclear, one theory is that the diminished platelet count is secondary to an immune-mediated response. This is supported by several of the patients having anti-platelet antibodies in their serum, response to immunosuppressive therapy, and increase in cell counts as the HEV infection resolved[37-39]. However, transient thrombocytopenia can be seen in a variety of inflammatory and infectious conditions, and further studies are needed to explore the underlying etiology given the rarity of this presentation.

Acute pancreatitis is another rare and poorly understood complication of HEV infection affecting only 6.2% of patients with acute HEV infection according to a study in Nepal[40]. Pancreatitis is more common, however, in patients with FHF, with one autopsy study finding pancreatitis in 44% of patients afflicted with FHF. It should be noted, however, that the study did not differentiate between different pathogens of viral hepatitis[41]. Interestingly, the few cases of HEV-related acute pancreatitis have almost exclusively been reported in India, where the virus is still endemic[42]. A single-center study in India by Raj *et al*[43] found that 2.1% of all patients with acute pancreatitis had acute HEV infection.

The pathophysiology of pancreatitis in HEV infection is poorly described in literature, though several theories have been postulated. One of the prevailing theories is that HEV virus is directly cytotoxic to pancreatic cells[44]. Other studies have hypothesized that swelling at the ampulla of Vater is caused by inflammation which inhibits secretion of pancreatic fluids[41]. One proposed cause is release of lysosomal enzymes from the liver which activate trypsin from trypsinogen and cause inflammation of the pancreas[40]. HEV-related pancreatitis is poorly understood, and further studies are warranted to elucidate the relationship between HEV infection and acute pancreatitis, as well as patient outcomes.

TREATMENT

Acute HEV infection usually does not require antiviral treatment, however, it should be considered in high risk patients or those with chronic infection. A small proportion of patients with acute HEV may progress to FHF or acute-on-chronic liver failure, particularly older men, pregnant women, and patients with underlying chronic liver disease. The most commonly used treatment in such cases is ribavirin, a guanosine analog[45]. Ribavirin has been shown to help clear the HEV virus and normalize liver enzymes[46,47]. The mechanism is not well understood, but is thought to deplete guanosine triphosphate pools, thus inhibiting HEV RNA replication. Though rare, ribavirin has been associated with hemolytic anemia, which can be severe in patients with underlying liver disease or chronic kidney disease[48].

Chronic HEV is most commonly seen in solid-organ transplant recipients, and the first step in management of these patients is to decrease the dose of immunosuppressive agents that target T-lymphocytes. Studies have shown that this step alone can lead to HEV clearance in 25%-33% of patients[49,50]. Pegylated interferon-alpha (PEG-IFN) should be avoided in patients with a history of solid organ transplants including heart, lung, pancreas, or kidney due to an increased risk of rejection. However, PEG-IFN can cautiously be used in patients with a history of liver transplantation, since the risk of rejection is lower[51,52]. Ribavirin is thought to be safe for use in the transplant population and therefore is the preferred agent. Retrospective studies have shown that 78-81% of patients with chronic HEV had undetectable HEV RNA in the serum 6 mo after completion of the ribavirin treatment course. This proportion increased to as high as 90% when treatment duration was prolonged in those who did not achieve sustained virologic response[53]. The regimen of choice is ribavirin 600 milligrams daily for 3 mo (unless longer course desired due to lack of sustained response). Similar to in hepatitis C (HCV) infection, some associations including the Grupo de Estudio de Hepatitis Virales (GeHEP) de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC) recommend a weight-adjusted dosing of ribavirin for treatment of HEV[54,55].

One of the limitations of ribavirin in the management of HEV is its potential for teratogenicity, given that pregnant patients are at increased risk of developing severe infection. However, a study by Sinclair *et al*[56] found no evidence for teratogenic effects in pregnant patients with HCV. The mortality rate of HEV infection in the third trimester of pregnancy is nearly 20%, so ribavirin should be considered in this population as organogenesis is generally complete prior to this phase of pregnancy. Additionally, severe hemolytic anemia is a potential complication of ribavirin, and patients should be monitored closely. Liver transplantation should be considered in patients that are progressing to liver failure despite appropriate management[56].

Sofosbuvir, the NS5B polymerase inhibitor used to treat HCV, has been a subject of investigation for the treatment of HEV. It has questionable efficacy as monotherapy for HEV, given the high relapse rates and incomplete initial response [57]. Studies have found mixed results regarding sofosbuvir/ribavirin combination therapy, with some showing efficacy in acute HEV infection and other showing inadequate response in solid-organ transplant patients with chronic infection [58-60]. Further studies and randomized clinical trials are needed to determine the proper treatment regimen and patient population best suited for these agents. A treatment flowchart for HEV infection is outlined in Figure 4.

VACCINATION

There is only one currently approved vaccine for HEV, which was first licensed in China in 2011[61]. The HEV vaccine has been found to be effective in establishing long-lasting immunity against HEV genotypes 1 and 4[62,63]. In the study, 48420 healthy subjects received three doses (given at 0, 1 and 6 mo) of the vaccine and 48420 received placebo. No patients in the vaccine group developed HEV infection after 12 mo, compared to 15 patients in the placebo group, giving the vaccine 100% efficacy[64]. A clinical trial is currently recruiting in China to assess the long term effectiveness of the vaccine (NCT05976594)[65]. Li *et al*[66] found that the HEV vaccine is effective against genotype 3 in rabbit models, however its efficacy in humans remains unclear. Additionally, Sridhar *et al*[67] demonstrated that the HEV vaccine is not effective in HEV-C due to antigenic divergence, however, identified HEV-C1 p241 peptides as a potential vaccine candidate against HEV-C infection.

A topic of interest in recent years has been vaccination of at-risk populations. Ji *et al*[68] established a proof of concept demonstrating that administering the HEV vaccine to a German population with high levels of pork consumption would result in an 80% reduction in human HEV cases. Immunosuppressed patients (*i.e.* organ transplant recipients) are at increased risk for developing chronic infection, and therefore may warrant extra consideration for vaccination. However, rabbit models have demonstrated that HEV vaccination following initiation of immunosuppressive agents only conferred partial immunity, which did not improve with additional or increased vaccine doses[69]. Pregnant women are also considered high risk, and a randomized control trial is currently underway assessing the efficacy of HEV vaccination in pregnant women in rural Bangladesh[70]. A phase II randomized clinical trial assessing the safety and immunogenicity of the HEV vaccine in pregnant patients in Pakistan is expected to reach completion in 2025 (NCT05808166)[71]. Given the evidence showing worse outcomes and accelerated progression of liver damage in patients with pre-existing liver disease, HEV vaccination should be considered in these individuals. A major limitation of the HEV vaccine trials is the exclusion of patients with chronic liver disease, necessitating further studies to assess vaccine efficacy in this group[26].

CONCLUSION

HEV infection is a common cause of acute hepatitis worldwide that is usually characterized by an acute, self-limited course of symptoms including anorexia, nausea and jaundice. It has been the causative agent of many outbreaks in developing nations in Africa, Asia, and Central America, but has also been increasing in prevalence in developed countries. Risk factors such as pregnancy and chronic liver disease have been associated with a more severe disease course and immunosuppression with chronic HEV infection. Though there is currently no Food and Drug Administration approved treatment for HEV, ribavirin has shown efficacy in many studies and is the most commonly recommended treatment. The recombinant HEV vaccination licensed in China is the only vaccine currently available for HEV, and its long-term efficacy as well as its safety in various populations is being studied. Further studies are needed to establish a guideline-based treatment regimen for HEV in order to decrease global morbidity, mortality, and healthcare burden.

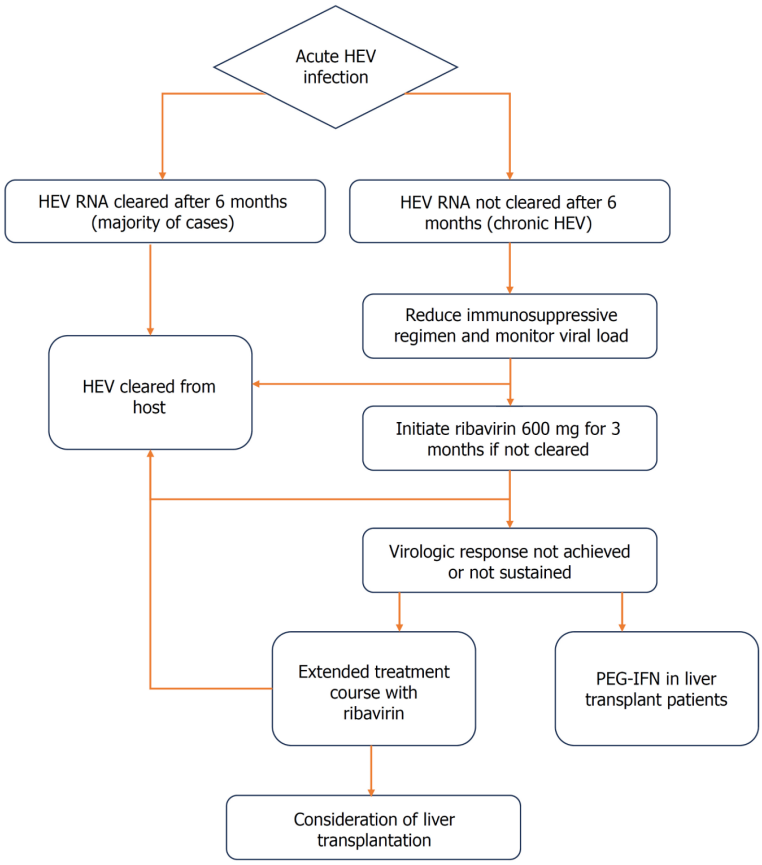

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Figure 4 Flowchart of treatment for hepatitis E virus infection.

FOOTNOTES

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ORIGINAL ARTICLE

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COVID-19 frequency and clinical course in children with asthma

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Abstract

BACKGROUND

The epidemic of severe acute respiratory syndrome coronavirus 2 infection, known as the coronavirus disease 2019 (COVID-19), has caused a global health concern. Since its emergence, numerous studies have focused on various clinical manifestations and outcomes in different populations. However, studies are ongoing as the consequences and impact of COVID-19 in children with chronic diseases such as asthma are controversial.

AIM

To fill this research gap by retrospectively evaluating the course, laboratory, and clinical findings of COVID-19 among 414 asthmatic children followed up from the pediatric allergy outpatient clinic and known to have had COVID-19.

METHODS

The data of 5510 patients over the age of 5 diagnosed with asthma in our hospital's data were retrospectively scanned with specific parameters using protocol numbers from the hospital filing system. The data included retrospective evaluation of pulmonary function test results before and after COVID-19, routine hematological and biochemical parameters, sensitization states (total IgE, specific IgE, and skin prick test results), and radiological (computed tomography) findings. To inquire about the course and symptoms of COVID-19, asthma patients or their parents were then called and evaluated with a questionnaire.

RESULTS

As a result of retrospectively scanning the data of 5510 asthma patients over the age of 5, it was determined that 414 (7.5%) patients had COVID-19. The mean age of 414 patients was 17.18 ± 4.08 (min: 6; max: 28) years. Two hundred and three of our 414 patients are male, and 211 are female. When their vaccination status was questioned, 21.5% were vaccinated. When the symptoms of our 290 patients were

questioned, it was stated that 59.0% had fever symptoms. The rate of using regular prophylactic asthma medications was 19%. The rate of using salbutamol in asthma was found to be 22%. The rate of patients using methylprednisolone was 1%. Emergency service admission was 17.2%, and hospitalization was found to be 4.8%. Leukopenia (< 4000) was found in 14.1% of patients, and 8.08% of our patients had neutropenia (< 1500). Lymphopenia (< 1500) was detected in 44.4% of patients, and lymphocytosis (> 4000) was found in 5.05% of patients. In 65% of our patients, the C-reactive protein value was elevated. A high aspartate aminotransferase and alanine aminotransferase value was detected in 3.2% and 5.4% of patients were found, respectively. 31% of patients had an elevated lactate dehydrogenase value. Typical radiological findings for COVID-19 were detected in 3/309 of patients.

CONCLUSION

According to our study, there is a correlation between the severity of COVID-19 and asthma symptoms and the course of the disease. However, it is worth noting that the retrospective nature of the study and the differences in sample size, age, and demographic characteristics between the two groups do not allow for an optimal comparison. Therefore, further investigation is needed to explore the relationship between COVID-19 and asthma, and it can be suggested that COVID-19 may trigger asthma attacks and asthma may impact the course of COVID-19.

Key Words: COVID-19; SARS-CoV-2, Children; Asthma; Exacerbation; Allergy

Core Tip: In our comprehensive retrospective study, we have made a noteworthy observation indicating a correlation between the severity of coronavirus disease 2019 (COVID-19) and the presence of asthma symptoms, which also appear to influence the course of the disease. These findings offer valuable insights into the potential interaction between COVID-19 and asthma. Given the complexity of this relationship and its possible implications for patient management, further in-depth investigations are warranted to elucidate the precise mechanisms and associations at play, aiming to improve our understanding and management of both conditions.

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INTRODUCTION

The epidemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, known as the coronavirus disease 2019 (COVID-19), has caused a global health concern. Since its emergence, numerous studies have focused on various clinical manifestations and outcomes in different populations. However, studies are ongoing as the consequences and impact of COVID-19 in children with chronic diseases such as asthma are controversial.

Asthma, a chronic inflammatory disease of the airways, is one of the most common non-communicable chronic childhood diseases among children. According to the American Centers for Disease Control and Prevention's (CDC) data, approximately 6.5% of children under the age of 18 in the United States have asthma[1]. Globally, it is estimated that 262 million people have asthma in 2019[2]. Children with asthma are often more susceptible to viruses that infect the respiratory tract, including common coronaviruses[3]. However, the relationship between asthma and a novel coronavirus, SARS-CoV-2, in the pediatric population has not yet been fully established.

This study aims to fill this research gap by retrospectively evaluating the course, laboratory, and clinical findings of COVID-19 among 414 asthmatic children followed up from the pediatric allergy outpatient clinic and known to have had COVID-19. We hope to shed light on the clinical manifestations, severity, and prognosis of COVID-19 in this specific pediatric population, helping develop targeted treatment strategies.

MATERIALS AND METHODS

In our study, the data of 5510 patients over the age of 5 diagnosed with asthma in our hospital's data were retrospectively scanned with certain parameters using protocol numbers from the hospital filing system. This study's approval was obtained from the Sakarya University Faculty of Medicine clinical research ethics committee (Decision No: E-71522473-050.01.04-128344-122).

The data included a retrospective evaluation of pulmonary function test results before and after COVID-19, sensitization states (total IgE, specific IgE, and skin prick test results), and radiological (computed tomography) findings.

Also, routine hematological and biochemical parameters of the patients including hemoglobin, leukocyte, neutrophil, lymphocyte, eosinophil, platelet counts, C-reactive protein (CRP), sedimentation rate, urea, aspartate aminotransferase (AST)-alanine aminotransferase (ALT), prothrombin time (PT), activated partial thromboplastin time (aPTT), D-dimer, lactate dehydrogenase (LDH) and finally total IgE values were evaluated. These values were collected retrospectively from our hospital filing system and laboratory evaluations performed within 2 wk of having COVID-19.

In order to inquire about the course and symptoms of COVID-19, 414/5510 (7.5%) asthma patients who had COVID-19 or their parents were then called and evaluated with a questionnaire.

Statistical analysis

Descriptive analyses were performed to provide information on the general characteristics of the study population. The Kolmogorov-Smirnov test was used to evaluate whether the distributions of numerical variables were normal. Accordingly, one-way ANOVA or Kruskal Wallis test was used to compare the numeric variables among three groups (for multiple comparisons of ANOVA and Kruskal Wallis tests, Sheffe and Dunn's test was used). Eta squared was calculated for the effect size of ANOVA or Kruskal Wallis test. The numeric variables were presented as mean ± standard deviation. The Chi-Square test compared categorical variables. Cramer V coefficient was calculated for the effect size of the Chi-Square test. Categorical variables were presented as a count and percentage. A P value < 0.05 was considered significant. Analyses were performed using SPSS statistical software (IBM SPSS Statistics, Version 22.0. Armonk, NY: IBM Corp.)

RESULTS

As a result of retrospectively scanning the data of 5510 asthma patients over the age of 5, it was determined that 414 (7.5%) patients had COVID-19. When their intra-familial contamination status was questioned, 36% stated that they had positive intra-familial transmission cases; the rest were not intra-familial transmission. The intra-familial transmission was highest in atopic patients. When their vaccination status was questioned, 21.5% were vaccinated. The mean age of 414 patients was 17.18 ± 4.08 (min: 6; max: 28) years. Two hundred and three of our 414 patients are male, and 211 are female. Three hundred and nine of our 414 patients could be reached by phone.

When the symptoms of our available 290 patients were questioned, it was stated that 59.0% had fever symptoms. Interestingly, one of our patients stated that he had a decrease in fever and was even admitted to the emergency department with the risk of hypothermia. Fatigue 39.0%; muscle pain 33.8%; headache 33.2%; cough 32.5%; sore throat 23%; joint pain 23%; shortness of breath 18.8%; runny nose 10.0%; nausea 9%; vomiting 6.8%; diarrhea 5%; loss of taste and smell 5.02% patients were reported to have symptoms. There was no significant difference among the rest of asthma group (others), vaccinated and atopic asthma patient groups, except for shortness of breath (Table 1; Figure 1).

The rate of using regular preventive/prophylactic asthma medications was 19%. The rate of using salbutamol in asthma attacks was found to be 22%. The rate of patients using prednol (methylprednisolone) is 1%. Emergency service admission was 17.2%, and hospitalization was found to be 4%. The number of days hospitalized patients stay in the hospital varies between 3-10 d. In addition, it was determined that one of our patients was hospitalized in the intensive care unit. A table shows the evaluation of therapeutic features in different patient groups (Table 2; Figure 2).

Upon inquiry about the general medications employed by our patients during their COVID-19 illness, the investigation yielded the subsequent findings. Out of the participants, 126 individuals utilized antipyretic-analgesic group drugs, 22 antiviral drugs, 18 leukotriene receptor antagonists, and 11 were treated with antibiotic group drugs. Furthermore, six patients opted for 2nd generation antihistamine group drugs, and two patients utilized antiemetic derivative drugs. Among the sample of 272 patients, 173 individuals (63%) reported needing medication during the COVID-19 period.

After investigating the recovery times of our patients, the findings showed that out of 309 patients, 272 (88%) could heal within 1-15 d. Only a small percentage of patients, three (0.9%), required a longer recovery time of 30 days. Additionally, two patients needed more extensive recovery times of 45 and 150 d, respectively.

According to these parameters, the hemogram values of 97 of our patients could be reached (Table 3). Leukopenia was found in 14/96 (14.6%) patients, and leukocytosis was found in 12.5%. Eight point three percent of our patients had neutropenia (< 1500), and 12.5% of patients had neutrophilia (> 7500). Lymphopenia (< 1500) was detected in 45.4% of patients, and lymphocytosis (> 4000) was found in 5.2% of patients. In our 6.2% of patients, elevated eosinophil (> 500) values (eosinophilia) were detected. 6.2% of patients had thrombocytopenia (< 150000); 1.03% of patients had thrombocytosis (> 450.000) (Table 4; Figure 3).

From the parameters of the coagulation system, the number of patients whose PT values were reached is 45. A high PT value (> 13.2) was detected in 11.1% of patients. The number of patients whose aPTT value was reached is 42. aPTT values were elevated (> 33.5) in 4.7% of our patients. D-dimer value was elevated (> 500) in 11.7% of our patients (Table 4; Figure 4).

When we look at the other biochemical parameters, the CRP value of 84 of our patients was reached. In 65% of our patients, the CRP value was elevated. High sedimentation value was detected in 14% of our patients. The number of patients whose AST and ALT values were reached is 92. An increased AST and ALT value was detected in 3.2% and 5.4% of patients, respectively. The number of patients whose LDH value was reached was 67, and 31.3% had an elevated LDH value. Total IgE values of 52 patients were reached. A high (> 150) total IgE value was found in 38.4% of patients (Table 4; Figure 4).

Table 1 Evaluation of clinical features in different groups of asthma patients

	Others[1] (*n* = 169)	Vaccinated patients (*n* = 57)	Atopic patients (*n* = 64)	*P* value	ES
Gender (male)	128/169 (47.6)	26/57 (45.6)	38/64 (59.4)	0.199	0.091
Fever	100/163 (61.3)	27/57 (47.4)	40/63 (63.5)	0.130	0.120
Fatigue	60/163 (36.8)	27/57 (47.4)	24/63 (38.1)	0.365	0.084
Headache	51/163 (31.3)	23/57 (40.4)	22/63 (34.9)	0.453	0.075
Muscle pain	62/163 (38)	17/57 (29.8)	18/63 (28.6)	0.296	0.093
Cough	53/163 (32.5)	24/57 (42.1)	17/63 (27)	0.205	0.106
Sore throat	41/163 (25.2)	15/57 (26.3)	10/63 (15.9)	0.280	0.095
Joint pain	43/163 (26.4)	11/57 (19.3)	13/63 (20.6)	0.452	0.075
Shortness of breath	23/163 (14.1)	17/57 (29.8)	14/63 (22.2)	**0.026**	0.160
Runny nose	19/163 (11.7)	5/57 (8.8)	5/63 (7.9)	0.653	0.055
Nausea	15/163 (9.2)	5/57 (8.8)	6/63 (9.5)	0.990	0.008
Vomiting	10/163 (6.1)	4/57 (7)	6/63 (9.5)	0.660	0.053
Diarrhea	9/163 (5.5)	4/57 (7)	2/63 (3.2)	0.624	0.057
Loss of taste and smell	6/163 (3.7)	6/57 (10.5)	3/63 (4.8)	0.148	0.119
Intra-familial contamination	59/164 (36)	15/57 (26.3)	31/64 (48.4)	**0.039**	0.151

[1]This column includes asthma patients who are neither atopic nor vaccinated.
Statistics were shown as *n*/total *n* (%). ES: Effect size (Cramer v coefficient).

Table 2 Evaluation of therapeutic features in different groups of asthma patients

	Others[1] (*n* = 169)	Vaccinated patients (*n* = 57)	Atopic patients (*n* = 64)	*P* value	ES
Preventive/prophylactic asthma medication usage	31/164 (18.9)	9/57 (16.1)	16/62 (25.8)	0.372	0.084
Salbutamol usage	34/164 (20.7)	11/57 (19.6)	20/63 (31.7)	0.169	0.112
Prednol usage	2/164 (1.2)	1/57 (1.8)	0/64 (0)	0.619	0.059
Emergency service admission	27/164 (16.5)	8/57 (14)	14/64 (21.9)	0.485	0.071
Hospitalization	7/164 (4.3)	3/57 (5.3)	4/64 (6.3)	0.866	0.038

[1]This column includes asthma patients who are neither atopic nor vaccinated.
Statistics were shown as *n*/total *n* (%). ES: Effect size (Cramer v coefficient).

Computed tomography (CT) reports of our 7/309 patients that may be associated with COVID-19 have been obtained. When we evaluated our patients' very few CT findings regarding COVID-19, findings that were shown as typical findings for COVID-19[4] were detected in 3 of our patients. The findings detected in the remaining patients were classified as findings in other viral pneumonia types that are not specific to COVID-19.

COVID-19 in asthmatic patients with atopy

When we look at patients with COVID-19, it was determined that 110/414 (26%) patients had atopy/sensitivity. In the filing system, inhalant allergy was found in 100 patients, food allergy in 4 patients, and inhalant and food allergy in 6 patients.

Considering that 64 patients could be reached by phone; in 48.4% of patients, intra-familial transmission was detected. In 41.6% of patients, it was transmitted from outside the family. 27% of our patients were vaccinated before contracting COVID-19. A higher incidence of intra-familial transmission was detected in these patients compared to the others.

Of 64 patients, 63.5% reported fever as a symptom, while 38.1% experienced fatigue and 34% had headaches. Muscle pain was reported by 28% of the patients, while 27% had a cough. Joint pain and shortness of breath were reported by 20% and 22% of patients, respectively; while sore throat and runny nose were reported by 15% and 7%, respectively. Vomiting was reported by 9% of patients, while 9% experienced nausea and 3.2% had diarrhea. Additionally, 4% of patients experienced loss of taste and smell (Figure 1B).

Table 3 Evaluation of various laboratory parameters in different groups of asthma patients

	Others[4], (*n* = 169)		Vaccinated patients, (*n* = 57)		Atopic patients, (*n* = 64)		*P* value	ES
	n	mean ± SD	*n*	mean ± SD	*n*	mean ± SD		
Age	269	16.57 ± 4.16[a]	57	18.86 ± 3.43[b]	64	15.77 ± 3.87[a]	**< 0.001**[1,3]	0.049
COVID-19 age	269	14.71 ± 4.09[a]	57	17.28 ± 3.55[b]	64	13.83 ± 3.75[a]	**< 0.001**[1,3]	0.063
Hemoglobin	68	13.13 ± 1.44	12	13.66 ± 1.57	17	12.96 ± 1.83	0.456[1]	0.017
Leucocyte	67	6803.79 ± 2825.88	12	6162.5 ± 2173.43	17	7068.24 ± 4155.47	0.903[2]	0.007
Neutrophil	67	4119.30 ± 2601.78	12	3563.33 ± 1721.86	17	3762.94 ± 2089.5	0.920[2]	0.008
Lymphocyte	68	1861.94 ± 939.88	12	1924.17 ± 1008.59	17	2503.53 ± 2908.44	0.915[2]	0.027
Eosinophil	67	141.91 ± 171.81	12	157 ± 149.52	17	146.29 ± 190.62	0.327[2]	0.001
Platelet	68	245864.71 ± 74049.74	12	273750 ± 77324.73	17	232352.94 ± 66343.75	0.368[2]	0.024
CRP	62	10.28 ± 20.13[a]	8	7.84 ± 5.34[a]	14	2.85 ± 2.41[b]	**0.016**[2,3]	0.025
Sedimentation	5	11 ± 7.52	2	5 ± 1.41			-	-
AST	63	25.58 ± 11.28	12	22.92 ± 5.23	16	27.06 ± 12.68	0.829[2]	0.011
ALT	63	19.13 ± 13.72	12	20.17 ± 14.83	16	20.06 ± 17.47	0.908[2]	0.001
Urea	61	21.5 ± 6.2	12	21.35 ± 7.18	16	22.45 ± 6.81	0.727[2]	0.004
PT	33	12.07 ± 1.24	4	11.65 ± 0.95	8	12.49 ± 1.25	0.512[1]	0.031
aPTT	31	28.87 ± 3.4	4	28.53 ± 1.97	7	28.96 ± 2.17	0.974[1]	0.001
D-Dimer	48	508.98 ± 953.33	9	334.78 ± 206.13	11	265.18 ± 128.16	0.647[2]	0.015
LDH	47	243.44 ± 88.75	8	202.59 ± 54.89	12	225.16 ± 87.56	0.312[2]	0.027
Total IgE	37	176.05 ± 252.36[a]	2	174.5 ± 144.96[a,b]	13	1127.04 ± 1325.03[b]	**0.009**[2,3]	0.274

[1]*P* values for one way ANOVA.
[2]*P* values for Kruskal Wallis test.
[3]According to the results of the pairwise comparison test, in addition to group statistics, there is no statistically significant difference between groups with the same superscript letter (a/a or b/b), but there is a statistically significant difference between groups with different superscript letters (a/b).
[4]This column includes asthma patients who are neither atopic nor vaccinated.
ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; aPTT: Activated partial thromboplastin time; COVID-19: Coronavirus disease 2019; CRP: C-reactive protein; LDH: Lactate dehydrogenase.

When questioning the regular use of preventive/prophylactic asthma medication during COVID-19; 25% of our patients felt the need to use asthma medications. While 31% of patients needed salbutamol, none of our patients required prednol (methylprednisolone). The number of patients admitted to the emergency department was 20.6%. 5.7% of our patients were hospitalized (Figure 2B).

Comparison of the findings of vaccinated and unvaccinated patients

Our total vaccinated patients were 57/290 (19.6%). Among the vaccinated patients, fever symptoms were found at 47.4%, fatigue at 47.4%, and the number of patients with shortness of breath was 29.8%. Among the unvaccinated patients, 59% of patients described fever symptoms. While 36% of our patients complained of fatigue, 16% complained of shortness of breath (Table 1).

The rate of using salbutamol among vaccinated patients was 19.6%. The number of patients admitted to the emergency department is 14%. We had a total of 5.3% vaccinated patients hospitalized, and the need to use prednol was necessary in only 1/57 of our patients (Table 2). The average recovery time of the vaccinated patients was calculated as 9.1 d.

The rate of using salbutamol among unvaccinated patients was 26%. The number of patients admitted to the emergency department was 19%. A total of 5% of patients were hospitalized among unvaccinated patients, and the need for prednol was necessary for 3/228 of our patients (Table 2). In addition, the average recovery time of unvaccinated patients was calculated as 6.2 d.

DISCUSSION

In a review that included eight retrospective studies and 2914 children with COVID-19, asthma is one of the most common causes of comorbidity[5]. In the report published by the American CDC in 2020, the prevalence of asthma

Table 4 Evaluation of pathologic laboratory parameters in different groups of our asthma patients

	Others[1], (*n* = 169)	Vaccinated patients (*n* = 57)	Atopic patients (*n* = 64)	*P* value	ES
Leukopenia (< 4000)	8/67 (11.9)	2/12 (16.7)	4/17 (23.5)	0.541	0.125
Leukocytosis (> 10000)	11/67 (16.4)	0/12 (0)	1/17 (5.9)	0.193	0.186
Neutropenia (< 1500)	6/67 (9)	1/12 (8.3)	1/17 (5.9)	1.000	0.042
Neutrophilia (> 7500)	10/67 (14.9)	0/12 (0)	2/17 (11.8)	0.447	0.147
Lymphopenia (< 1500)	29/68 (42.6)	5/12 (41.7)	10/17 (58.8)	0.470	0.125
Lymphocytosis (> 4000)	3/68 (4.4)	0/12 (0)	2/17 (11.8)	0.475	0.152
Eosinophilia (> 500)	4/67 (6)	1/12 (8.3)	1/17 (5.9)	1.000	0.033
Thrombocytopenia (< 150000)	5/68 (7.4)	0/12 (0)	1/17 (5.9)	0.832	0.099
Thrombocytosis (> 450000)	1/68 (1.5)	0/12 (0)	0/17 (0)	1.000	0.067
Elevated CRP (> 3)	42/62 (67.7)	7/8 (87.5)	6/14 (42.9)	0.090	0.245
Elevated sedimentation (> 20)	1/5 (20)	0/2 (0)	-	1.000	0.258
Elevated AST (> 50)	2/63 (3.2)	0/12 (0)	1/16 (6.3)	1.000	0.097
Elevated ALT (> 50)	3/63 (4.8)	1/12 (8.3)	1/16 (6.3)	1.000	0.054
Elevated urea (> 45)	0/61	0/12	0/16	-	-
Elevated PT (>13.2)	3/33 (9.1)	0/4 (0)	2/8 (25)	0.261	0.221
Elevated aPTT (> 33.5)	2/31 (6.5)	0/4 (0)	0/7 (0)	1.000	0.133
Elevated D-dimer (> 500)	6/48 (12.5)	1/9 (11.1)	1/11 (9.1)	1.000	0.039
Elevated LDH (> 248)	15/47 (31.9)	2/8 (25)	4/12 (33.3)	1.000	0.052
Elevated total IgE (> 150)	10/37 (27)	1/2 (50)	9/13 (69.2)	**0.019**	0.376

[1]This column includes asthma patients who are neither atopic nor vaccinated.
Statistics were shown as *n*/total *n* (%). ES: Effect size (Cramer v coefficient).

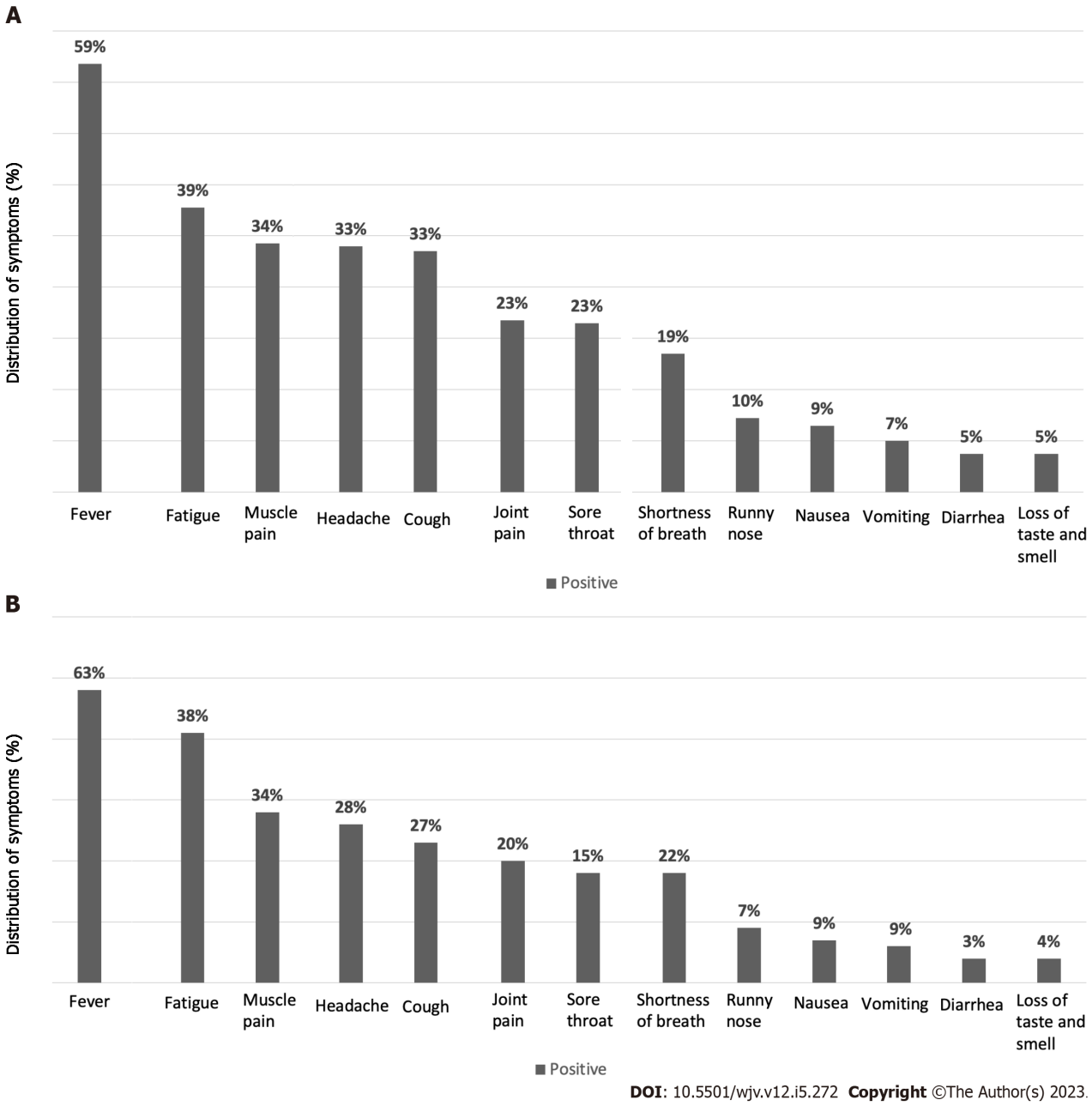
among those who had COVID-19 was reported as 5.8%[1]. In another study, which included 1802 patients with COVID-19, the rate of asthmatic patients was 7.8%[6]. It is known that the prevalence of asthma among COVID-19 patients is less common than in the general population[7,8].

After scanning the data of 5510 asthma patients over the age of 5, it was determined that 414 (7.5%) patients had COVID-19. In another study involving 43000 SARS-CoV-2 positive patients, asthma was reported as the most common comorbidity, with a prevalence of 10.2%[9]. In a study that involved 91 centers caring for approximately 133000 children with asthma, only 13/91 (14%) of the participating centers reported suspected cases of COVID-19 in children with asthma [10].

When we look at the vaccination rate of our patients, the vaccination rates among our patient group for individuals aged 12 and above and those aged 18 and above were determined to be 21.5% and 12.7% respectively. The vaccination rates in our study were lower than the current data because asthmatic patients in the childhood age group (17.18 ± 4.08 years) were included in the study, and the age limit for vaccination was lowered to 12 years in our country much later. In addition, as another factor, vaccine hesitancy at the time of the first use of vaccines is a known phenomenon that is still effective today. In a study that included 637 parents with children between 12 and 15 in the United States, vaccine hesitancy against COVID-19 vaccines was found in almost 1/3 of the parents[11]. A study conducted in our country stated that parents who were hesitant towards childhood vaccines also had a negative attitude toward COVID-19 vaccines[12]. In addition, in a study conducted with the parents of children with asthma in 2020, 19% of the participants (*n* = 309) stated that they did not consider vaccinating their children against COVID-19[13].

In our study, fever complaints were found at a rate of 59%, while this rate was lower in the literature compared to ours. In a study including 54 pediatric COVID-19 patients with asthma, complaints of fever were reported at a rate of 27.5%[5]. In a study comparing asthmatic COVID-19 patients and non-asthmatic COVID-19 patients, the rate of fever symptoms was 37.3% in the asthmatic group. Still, no significant difference was found between the two groups ($P = 0.55$)[7]. These data suggest that the rate of fever complaints in our study is higher than in other studies documented in the literature. When comparing vaccinated patients and unvaccinated patients regarding fever symptoms, no significant difference was observed between the two groups ($P = 0.012$).

The number of patients with cough complaints was found to be 33%. In a study including 54 COVID-19-positive asthma patients, the rate of cough (59.3%) was higher than in our study. In addition, the same study reported a significant difference in cough symptoms between the two groups with and without asthma ($P = 0.002$)[14]. In a study including 60 hospitalized asthma patients, it was reported that all asthma patients with COVID-19 (*n* = 10) had cough symptoms[15].



DOI: 10.5501/wjv.v12.i5.272

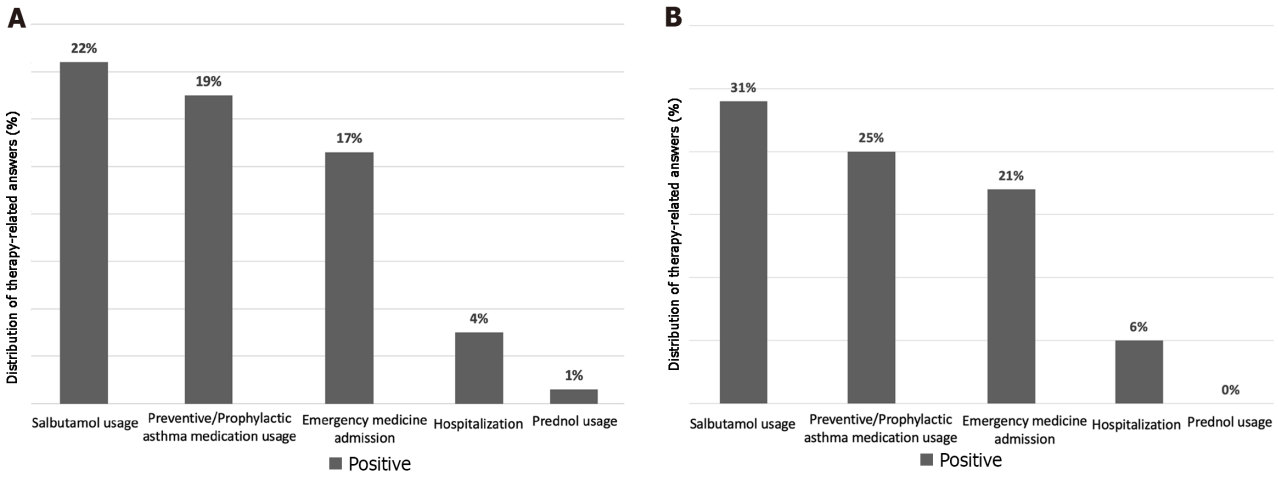
Figure 1 Symptom distributions of all patients. A: Distribution of symptoms according to the questions asked of patients and their answers; B: Distribution of symptoms according to the questions asked of patients with atopic asthma.

In our study, no significant difference was detected between the vaccinated and unvaccinated patient groups regarding cough complaints (P = 0.205).

When we look at the complaint of fatigue, the rate of 39% in our study was found to be higher compared to the literature. In one study, the rate of fatigue among COVID-19-positive patients with asthma was 16.9%[7]. Another study reported this rate as 16.7%[5].

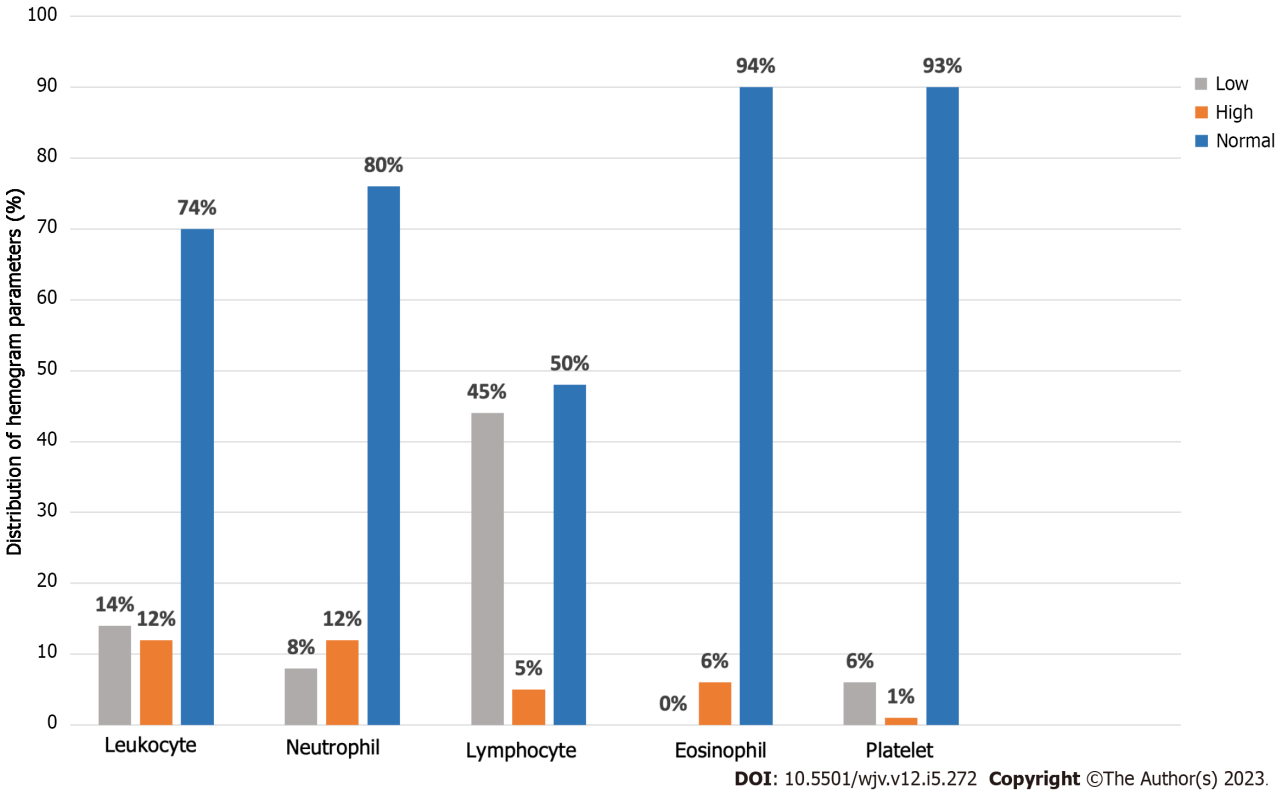
When we evaluated our vaccinated and unvaccinated patients in terms of symptom severity, it was found that 47% of vaccinated and 63% of unvaccinated patients had fever. It can be said that being vaccinated provides a 16% decrease in fever complaints, which seems significant (P = 0.008). When we look at fatigue complaints, vaccinated patients complained of fatigue 10% more often than non-vaccinated patients, a difference that can be considered significant (P = 0.002). In addition, dyspnea is 12% more common in vaccinated patients compared to unvaccinated patients (P = 0.041). When we look at the studies published in the literature, it has been reported that being vaccinated leads to a significant decrease in the frequency of symptoms, unlike the results we found. So, in a report published by the CDC, it was reported that vaccinated children had 60% fewer symptoms than those who were not vaccinated[16].

In our study, when the rate of asthma medication use was examined, we observed a rate of 21.2%. This rate was found to be lower compared to other studies in the literature. In a study conducted by Metbulut *et al*[5], the rate of asthma medication use was reported as 42.7%. When considering the rates of salbutamol usage, we found a rate of 22%, which was higher than that reported in other studies in the literature. In a study conducted by Gaietto *et al*[7], the rate of



DOI: 10.5501/wjv.v12.i5.272

Figure 2 Asthma control parameters. A: Distribution of therapy-related answers to the questions asked to patients with asthma; B: Distribution of therapy-related answers to the questions asked to asthma patients with atopy (atopic/allergic asthma).

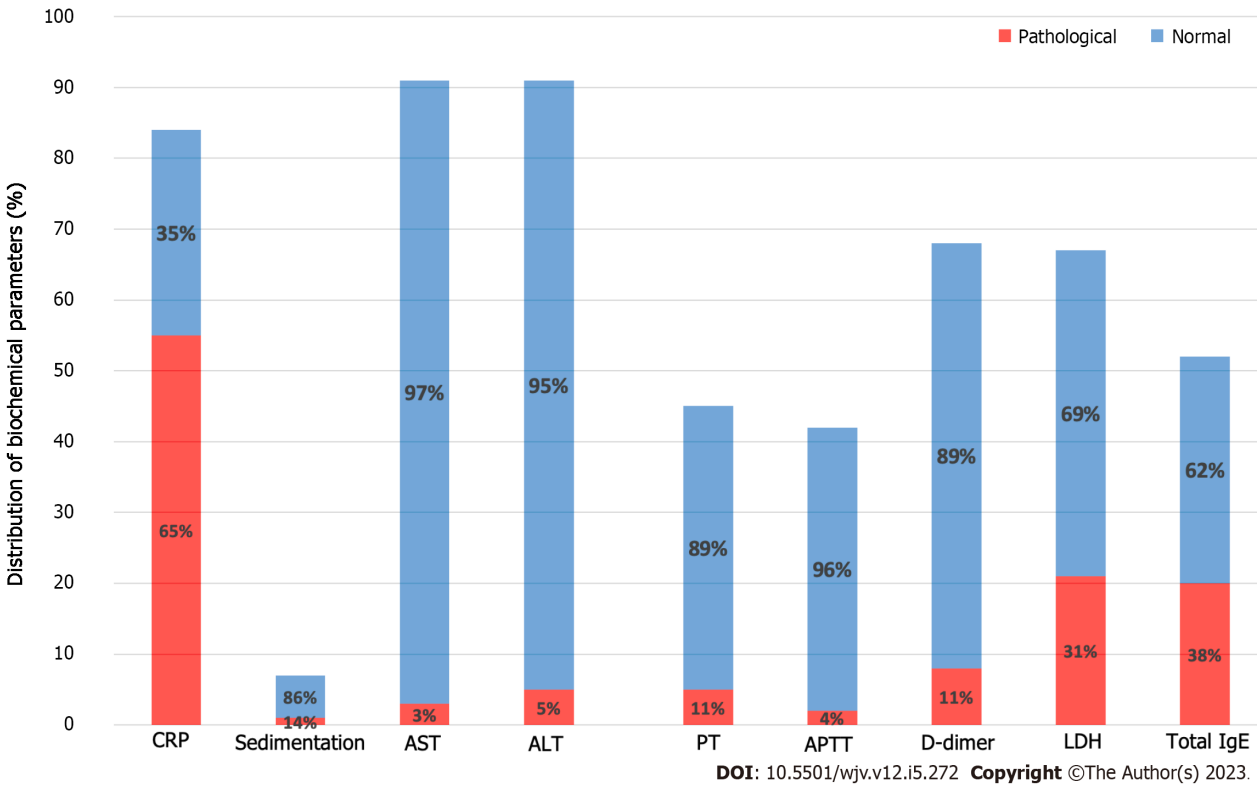


DOI: 10.5501/wjv.v12.i5.272

Figure 3 Distribution of hemogram parameters.

Salbutamol usage among asthmatic COVID-19 patients was 17.6%. As expected, when regular maintenance medications are not used, asthma attacks tend to occur more frequently, leading to an increased usage of rescue medication, such as beta-agonists. No significant difference was observed in the asthma medication usage rate between vaccinated and unvaccinated patients (P = 0.957).

In our study, the rate of emergency department visits was determined to be 17.2%. Indeed, this rate appears to be similar to other studies in the literature. In one study, the rate of emergency department visits among asthmatic COVID-19 patients was 13.4%[7]. Another study demonstrated that implementing lockdown measures during COVID-19 significantly reduced the rate of emergency department visits in asthmatic patients[17]. The rate of methylprednisolone usage among our patients was determined to be 1%. As emergency department visits decrease, the usage rate of oral steroids, usually required during attacks, is also found to decrease. Vaccinated patients had 6% fewer emergency department visits than unvaccinated ones, but this difference was not statistically significant.



DOI: 10.5501/wjv.v12.i5.272

Figure 4 Distribution of biochemical parameters.

Hospitalization rates were found to be 4% in our study. Indeed, this rate was similar to the rate (4.9%) in the study conducted by Gaietto *et al*[7]. Furthermore, in this study and other studies, asthma is a risk factor for hospitalization in SARS-CoV-2 infection[7,9]. In one study, the prevalence of asthma was reported as 34.2% in 34 hospitalized COVID-19 patients, and it was stated that COVID-19 did not show a serious course in asthma patients and required a lower level of care compared to other patients[18]. No significant difference was detected in terms of hospitalization between the vaccinated and unvaccinated patients (P = 0.692).

When examining the laboratory values of our patients, it was found that 14% exhibited leukopenia. While 10/14 (71%) of these patients had values in the normal range before COVID-19, a significant decrease was found in leukocyte counts during the period they had COVID-19. High leukocyte values were detected in one of our patients before contracting COVID-19. Based on these data, it appears that SARS-CoV-2 infection causes changes in patients' leukocyte counts. Leukopenia may be a common finding among patients with COVID-19, and a decrease in leukocyte count may inform the severity and course of the infection. However, it should be evaluated together with other clinical and laboratory data. In addition, in an article including 184 patients, leukopenia was found in 58 (34%) of the patients, but it is reported that only children with COVID-19 were screened in this article[14]. In a study comparing ten asthmatic patients with COVID-19 and 25 non-asthmatic patients, no significant difference was found in leukocyte counts[18]. No significant difference was observed in leukopenia frequency between our vaccinated patients and the unvaccinated patient group (P = 0.92).

When we look at our patients with leukocytosis (12%), the leukocyte count of only 23% of our patients was within the normal range before SARS-CoV-2 infection. There was no decrease in the rest of our patients, and an increase in leukocyte counts was detected before the infection. As a result, it was determined that the leukocyte count was not within the normal range and was already high in the majority of patients with leukocytosis in the period before SARS-CoV-2 infection. This situation requires careful evaluation of the effect of SARS-CoV-2 infection on leukocyte count, and the general clinical status and laboratory results of these patients should be evaluated together. In another article, it was reported that leukocytosis was detected in 194/610 (32%) pediatric moderately severe COVID-19 patients and 4/16 (25%) patients with severe COVID-19[19]. In another study comparing asthmatic patients with COVID-19 and patients without asthma, it was found that there was no significant difference between the two groups in terms of leukocyte values (P = 0.675)[20].

The number of patients with lymphopenia in our study was 44 out of 97 (45.3%), which is higher than other studies in the literature. In a study evaluating 66 COVID-19 patients aged between 6 and 17, lymphopenia was only observed in 2 out of 66 patients (3%)[21]. Another study, which included 486 hospitalized patients with confirmed SARS-CoV-2 infection, reported a prevalence of lymphopenia in 21% of the patients[22]. In a study comparing SARS-CoV-2 positive asthmatic patients (n = 54) with non-asthmatic patients (n = 162), no significant difference in serum lymphocyte levels was reported between the two groups (P = 0.263)[5].

When evaluating patients with neutropenia in our study, we observed neutropenia in only 8% of patients. Our findings appear to be consistent with the study conducted by Üzel *et al*[23]. In this study involving 59 patients, neutropenia was reported in 5 out of 59 patients (8.5%)[23]. However, it is worth noting that this study, unlike ours, specifically included

only children with COVID-19. Similar to our study, in a study comparing SARS-CoV-2 positive patients with and without asthma, no significant difference was found between the two groups in terms of serum neutrophil counts (P = 0.379)[5]. Another study comparing COVID-19 patients with and without asthma did not observe a significant difference between the two groups (P = 0.810)[20].

It can be stated that the platelet values of our patients yielded similar results compared to other studies in the literature. In our study, thrombocytopenia was observed in 6% of our 97 patients, while in a study conducted in Türkiye with 633 included patients, this rate was reported as 2%[24]. Furthermore, in the same article, the number of patients with thrombocytosis was 56 out of 633 (8.8%), whereas in our study, this rate was found to be 1% and represented a much smaller number of patients (n = 1)[24]. In another study comparing SARS-CoV-2 positive patients with and without asthma, no significant difference was found in platelet count (P = 0.480)[5].

When examining the CRP values of our patients, a significant elevation was observed in CRP levels in 54 out of 84 patients (65%). This rate is higher compared to other studies in the literature. In a study involving 633 patients, this rate was reported as 20%. The authors also noted that elevated CRP and other inflammatory markers may be associated with the severity of COVID-19[24]. In another study comparing SARS-CoV-2 positive patients with and without asthma, no significant difference was found in CRP values between the two groups (P = 0.523).

When evaluating LDH values, we found an elevation in LDH levels in 31% of our patients. This result is similar to the findings reported by Üzel *et al*[23]. Their study reported LDH elevation in 37.3% (n = 22) of the 59 symptomatic patients [23]. Another study compared LDH values between COVID-19 patients with asthma (n = 27) and without asthma (n = 42). This study found a significant difference in LDH values between the two groups (P = 0.035)[20].

When examining the D-dimer values of our patients, an elevation in D-dimer levels was observed in 8 out of 68 patients (11%). In a study involving 470 patients, D-dimer elevation was observed in 84 individuals (17.9%)[24]. Additionally, another article has linked elevated D-dimer levels and fibrinogen degradation products with COVID-19 mortality[25].

Comparison of symptoms in patients with and without atopy

When comparing the severity of COVID-19 in our patients with atopy and the other (nonatopic) group, we found that cough was observed in 27% of patients with atopy, while it increased to 32.5% in the other group; however, this difference was not statistically significant (P = 0.205). Fatigue was reported to be 1% more in patients with atopy compared to the other group. However, muscle pain was 10% more in other patients without atopy. Nevertheless, these two symptoms did not significantly differ (P = 0.365 and P = 0.296). Based on these findings, it is challenging to claim that systemic symptoms are a significant marker in patients with atopy. However, according to a study, the milder symptoms of COVID-19 in patients with atopy compared to those without atopy may be attributed to the hyperactivation of T cells in individuals with allergies[26]. Additionally, it is known that individuals with allergies who contract COVID-19 tend to have a milder course of the disease compared to those without allergies[27].

When considering the need for salbutamol, patients with atopy felt 11% more need than the other group; this difference was not statistically significant (P = 0.169). In our study, children with atopic asthma used asthma medications [inhaled corticosteroid (ICS)] 7% more than other group. However, this difference was not statistically significant (P = 0.372). In light of this information, it can be stated that patients with atopy experience an increase in asthma attacks when they contract SARS-CoV-2 infection. However, some studies in the literature have reported findings in favor of a decrease, rather than an increase, in asthma attacks during COVID-19, attributing the decrease in attack frequency to reduced exposure to allergens and outdoor inhaler risk factors for asthmatic children nationwide due to the precautions taken during the COVID-19 pandemic[26,28]. In our study, being atopic was suggested as a risk factor for triggering asthma attacks when children with atopy contracted SARS-CoV-2. When examining hospital admissions, those with atopy had 5% more emergency department visits compared to other group; however, this difference was not statistically significant (P = 0.485). Table 2 shows that the rates of emergency department visits and hospitalizations were higher but statistically insignificant in the atopic patients compared to the other group. Furthermore, it has been reported that COVID-19 does not progress to severe illness in patients with atopic asthma[29]. According to another study, overall hospital admission rates during the COVID-19 pandemic have dramatically decreased, but specific information about patients with atopic asthma was not shared in that study[29]. Another study reported that the rate of emergency department visits due to asthma decreased by 80% in 2020 compared to 2019 and 2018. Still, no patient atopic status data was provided[17].

When examining the biochemical and hematological parameters during the period of COVID-19 in patients with atopy and the other (nonatopic) group, it was found that patients with atopy had 10% less leukocytosis compared to the other group; however, this difference was not statistically significant (P = 0.193). In patients with atopy, a 12% higher rate of leukopenia was observed compared to the other group; however, we believe this difference is not significant (P = 0.541). In atopic children, elevated CRP values were found to be 25% less compared to the other group, and this difference was not statistically significant (P = 0.090). While lymphopenia, leukopenia, and elevated CRP values are known to be present in children with COVID-19, studies emphasize that these laboratory values are not specific to COVID-19[19].

Hospitalized vs non-hospitalized patients

When examining the symptoms of our patients who required hospitalization and experienced severe COVID-19, the most common complaints were fever and fatigue, with a prevalence of 57% and 40.6%; respectively. This was followed by headache and cough, with a prevalence of 35% and 33.6%; respectively. These data become more significant when comparing the group of hospitalized patients with those who were not. It was found that hospitalized patients had 23% more cough symptoms, but it was not statistically significant (P = 0.07). Hospitalized patients were seen to use 60% more salbutamol than non-hospitalized patients, and this difference was statistically significant. A positive correlation exists

between hospitalization and salbutamol use (P = 0.001). Hospitalized patients presented to the emergency department 34% more frequently; this difference was significant (P = 0.001). Additionally, a positive correlation exists between hospitalization and admission to the emergency department (P < 0.05). Hospitalized patients used methylprednisolone 13% more and asthma medications (ICS) 55% more compared to non-hospitalized patients, and these differences were statistically significant (P = 0.03 and 0.002, respectively).

When examining the acute phase reactants of our patients, elevated CRP levels were detected in 66%. These CRP values appear to be higher than in another study that included children with severe COVID-19 but are consistent[19]. Furthermore, leukopenia was observed in 17.3%, while leukocytosis in 7.3%. Neutropenia was found in 7.6%, and lymphopenia was found in 47.6%. Additionally, one patient had lymphocytosis and one patient had eosinophilia. Moreover, D-dimer elevation was observed in 11%, and LDH elevation was observed in 30%. D-dimer levels in the context of asthma and COVID-19 in children are crucial due to their association with coagulation activity and potential thrombotic complications. Increased D-dimer levels can function as indicators of heightened coagulation and fibrinolysis processes, potentially increasing the susceptibility of individuals to cerebrovascular events, including stroke[30]. Furthermore, research findings have indicated a notable correlation between D-dimer levels and the severity of the disease[31].

As many studies have indicated, asthma exacerbations did not increase in children during the COVID-19 period, and the implemented precautions and reduced allergen exposure have been reported to lead to a decrease in asthma symptoms[28,32]. According to our study, there is a correlation between the severity of COVID-19 and asthma symptoms and the course of the disease. However, it is worth noting that the retrospective nature of the study and the differences in sample size, age, and demographic characteristics between the two groups do not allow for an optimal comparison. Hospitalizations in children due to COVID-19 may increase asthma exacerbations, and asthma, which is shown as a risk factor in some data in the literature, is of importance[33]. Therefore, further investigation is needed to explore the relationship between COVID-19 and asthma, and it can be suggested that COVID-19 may trigger asthma attacks and asthma may impact the course of COVID-19.

Comparison of the findings of vaccinated and unvaccinated patients

When evaluating the symptom severity of the vaccinated and other (unvaccinated) group, it was found that 47% of vaccinated patients experienced fever. In comparison, the rate of fever complaints among the other group was 61%. It can be said that getting vaccinated resulted in a 14% decrease in fever complaints, which appears insignificant (P = 0.130). Regarding fatigue, vaccinated patients reported 11% more fatigue compared to the other group, but this difference is not significant (P = 0.365). Shortness of breath appears to be 15% more frequent in vaccinated patients compared to the other group. When examining the studies published in the literature, it is reported that vaccination leads to a significant decrease in symptom frequency, contrary to our findings. In fact, according to a report published by the CDC, vaccinated children experience the disease with 60% fewer symptoms compared to the unvaccinated[16].

As observed in Table 2, it can be seen that the rate of emergency department visits was lower in the vaccinated patients compared to atopic and other groups. Moreover, when we look at the control of asthma symptoms and hospitalization, no significant difference was observed between our study's groups of patients (vaccinated, atopic, and other [neither vaccinated nor atopic] group). Consistent with the findings of our study, a study conducted by Grandinetti *et al*[9] did not find any evidence supporting the hypothesis that COVID-19 vaccination exacerbates asthma attacks in asthmatic children. In our study, consistent with the previous findings, vaccinated patients experienced asthma exacerbations at a similar rate compared to the other group, and this difference did not appear to be statistically significant. The same study recommended deferring COVID-19 vaccination in patients with uncontrolled asthma until their clinical condition improves. A case study reported that an asthma patient who received the BNT162b2 (Pfizer-BioNTech) vaccine experienced an asthma exacerbation[34]. However, the authors noted that this study represents a single case, and further research is needed to conclude the general population.

Final a few points about the pathophysiology of COVID-19 and asthma

For a better understanding of the results of the study and the discussion, it would be appropriate to emphasize a few points about the pathophysiology of COVID-19 and allergic/non-allergic asthma diseases.

It is well known that asthma patients show reduced production of the antiviral interferon and lower ACE-2 expression. This is probably because ACE-2 expression is inversely correlated with type 2 (Th2: T helper 2) cytokine levels in atopic/allergic asthmatics. However, severe COVID-19 shows strong type I interferon expression early on. Consequently, this is inconsistent with the pathophysiology of COVID-19 disease development in asthma patients with a worse prognosis[8,27,29].

Although the impact of non-allergic and allergic asthma on the course of COVID-19 is often discussed in detail in the literature. It is reported that the prognosis of COVID-19 cases with common allergic diseases (atopic asthma, allergic rhinitis, atopic eczema, etc.) are not severe. Thus, the shift of the immune system to the Th2 phenotype in these patients may indicate a favorable balance in the pathogenesis of COVID-19 development[8,27,29].

CONCLUSION

According to our study, there is a correlation between the severity of COVID-19 and asthma symptoms and the course of the disease. However, it is worth noting that both the retrospective nature of the study and the differences in sample size, age, and demographic characteristics between the two groups do not allow for an optimal comparison. Therefore, further

investigation is needed to explore the relationship between COVID-19 and asthma, and it can be suggested that COVID-19 may trigger asthma attacks and asthma may impact the course of COVID-19.

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ARTICLE HIGHLIGHTS

Research background

Studies are ongoing as the consequences and impact of coronavirus disease 2019 (COVID-19) in children with chronic diseases such as asthma are controversial.

Research motivation

The consequences of COVID-19 in children with asthma are controversial.

Research objectives

This study aims to fill this research gap by retrospectively evaluating the course, laboratory, and clinical findings of COVID-19 among 414 asthmatic children followed up from the pediatric allergy outpatient clinic and known to have had COVID-19.

Research methods

The data of 5510 patients over the age of 5 diagnosed with asthma in our hospital's data were retrospectively scanned with certain parameters using protocol numbers from the hospital filing system.

Research results

As a result of retrospectively scanning the data of 5510 asthma patients over the age of 5, it was determined that 414 (7.5%) patients had COVID-19. The mean age of 414 patients was 17.18 ± 4.08 (min: 6; max: 28) years. 203 of our 414 patients are male, and 211 are female.

Research conclusions

According to our study, there is a correlation between the severity of COVID-19 and asthma symptoms and the course of the disease.

Research perspectives

Further investigation is needed to explore the relationship between COVID-19 and asthma, and it can be suggested that COVID-19 may trigger asthma attacks, and asthma may impact the course of COVID-19.

FOOTNOTES

Author contributions: Özata MC, Dikici Ü and Özdemir Ö designed the research; Özata MC and Dikici Ü performed the research; Dikici Ü and Özdemir Ö contributed analytic tools; Özata MC and Özdemir Ö analyzed the data; Özata MC, Dikici Ü and Özdemir Ö wrote the paper.

Institutional review board statement: This study's approval was obtained from the Sakarya University Faculty of Medicine clinical research ethics committee (Decision no: E-71522473-050.01.04-128344-122).

Informed consent statement: All study participants or their legal guardians, provided informed written consent before enrollment.

Conflict-of-interest statement: The authors have no conflict to disclose.

Data sharing statement: The data supporting this study's findings are available on request from the corresponding author.

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

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ORIGINAL ARTICLE

Retrospective Cohort Study

Use of inflammatory markers as predictor for mechanical ventilation in COVID-19 patients with stages IIIb-V chronic kidney disease?

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Abstract

BACKGROUND

Studies have shown elevated C-reactive protein (CRP) to predict mechanical ventilation (MV) in patients with coronavirus disease 2019 (COVID-19). Its utility is unknown in patients with chronic kidney disease (CKD), who have elevated baseline CRP levels due to chronic inflammation and reduced renal clearance.

AIM

To assess whether an association exists between elevated inflammatory markers and MV rate in patients with stages IIIb-V CKD and COVID-19.

METHODS

We conducted a retrospective cohort study on patients with COVID-19 and stages IIIb-V CKD. The primary outcome was the rate of invasive MV, the rate of noninvasive MV, and the rate of no MV. Statistical analyses used unpaired *t*-test for continuous variables and chi-square analysis for categorical variables. Cutoffs for variables were CRP: 100 mg/L, ferritin: 530 ng/mL, D-dimer: 0.5 mg/L, and lactate dehydrogenase (LDH): 590 U/L.

RESULTS

290 were screened, and 118 met the inclusion criteria. CRP, D-dimer, and ferritin were significantly different among the three groups. On univariate analysis for invasive MV (IMV), CRP had an odds ratio (OR)-5.44; ferritin, OR-2.8; LDH, OR-7.7; D-dimer, OR-3.9, (P < 0.05). The admission CRP level had an area under curve-receiver operator characteristic (AUROC): 0.747 for the IMV group (sensitivity-80.8%, specificity-50%) and 0.663 for the non-IMV (NIMV) group (area under the curve, sensitivity-69.2%, specificity-53%).

CONCLUSION

Our results demonstrate a positive correlation between CRP, ferritin, and D-dimer levels and MV and NIMV rates in CKD patients. The AUROC demonstrates a good sensitivity for CRP levels in detecting the need for MV in patients with stages IIIb-V CKD. This may be because of the greater magnitude of increased inflammation due to COVID-19 itself compared with increased inflammation and reduced clearance due to CKD alone.

Key Words: Coronavirus disease 2019; Chronic kidney disease; Inflammatory markers; C-reactive protein; Invasive mechanical ventilation; Non-invasive mechanical ventilation

Core Tip: Our study demonstrates a positive correlation between the levels of inflammatory markers, including C-reactive protein, ferritin, and D-dimer, and the rate of invasive and non-invasive mechanical ventilation (MV) among coronavirus disease 2019 patients with chronic kidney disease (CKD), suggesting that these biomarkers are clinically useful to predict the need for MV in the CKD population.

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INTRODUCTION

A new variant of coronavirus lead to the pandemic of 2019 and was described as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Initially assumed to be a pathogen affecting the respiratory system, its effects have now been shown to be widespread affecting multiorgan infection and disease manifestation. With more than six million admissions and more than 1 million deaths, coronavirus disease 2019 (COVID-19) continues to be an infection with ongoing global concern. As we continue to discover the multitude of pathologies caused by the virus in different organ systems, the various associations and interactions between COVID-19 and existing chronic diseases slowly come to light. It was a sudden increase in the utility of inflammatory biomarkers, as they served as useful indicators of the severity of the underlying disease process. Severe COVID-19 disease is characterized by a hyperinflammatory condition, with multiorgan involvement due to a cytokine storm[1]. Multiple organ specific and nonspecific markers have been studied. Cardiac troponins, brain natriuretic peptide and multiple other markers have been shown to predict outcome in patients with and without cardiovascular disease[2,3]. Similar inflammatory markers including cytokines, including interleukin-6 (IL-6) and C-reactive protein (CRP), have been validated in multiple studies to help predict the severity of disease and the need for mechanical ventilation (MV)[4-6]. Studies have shown baseline elevation in these same inflammatory markers in patients with chronic kidney disease (CKD) alone, due to a chronic inflammatory milieu in chronic kidney disease and reduced renal clearance of these inflammatory markers[7]. Currently, the clinical utility of these inflammatory markers to predict the need for MV among patients with COVID-19 and underlying CKD is unclear. We aimed to assess if elevations in inflammatory markers can similarly predict the rate of invasive and non-invasive MV (IMV) (NIMV) among COVID-19 patients with CKD.

MATERIALS AND METHODS

Study design and participants

We conducted a retrospective single-center cross-sectional study of hospitalized patients between Dec 1, 2019, to Jan 1, 2022, at a 329-bed community teaching hospital in central Massachusetts. In order to be recruited into the study participants had to meet the inclusion criteria. Inclusion criteria: (1) Inpatients admitted with clinical symptomatology and subsequently diagnosed with SARS-CoV-2 infection with polymerase chain reaction test; (2) Age > 18 years; (3) Patients with history of stages IIIb-V CKD (estimated glomerular filtration < 45 cc/min as per National Kidney Foundation guidelines); and (4) Patients with documented inflammatory markers within 24 h of admission to the

hospital. Exclusion criteria included: (1) Pregnant patients; (2) Patients who had a history of renal transplantation; (3) Patients who required renal replacement therapy; and (4) Patients who failed to meet the inclusion criteria or if the required information could not be collected. The data was obtained by reviewing medical records, including demographic information, past medical history, medications, labs, and hospitalization course. Two independent physicians were involved with acquiring the data. All patient details were anonymized. Preformed proforma was used to acquire the study details such as age, sex, vaccination status, comorbidities such as hypertension, diabetes mellitus, chronic liver disease, chronic obstructive pulmonary disease, coronary artery disease, congestive heart failure and the use of medications such as steroids or remdesivir.

Exposure and outcomes

The primary endpoints measured included the rate of IMV, the rate of NIMV, and the rate of no requirement of mechanical ventilatory support (no-MV). As per American Thoracic Society guidelines, IMV was defined as intubation and provision of mechanical ventilatory support for respiratory failure. NIMV included bi-level positive airway pressure, high-flow oxygen, and continuous positive airway pressure support. No MV was defined as requiring oxygen *via* nasal cannula, oxymizer support, or those who did not require any oxygen supplementation. We assessed the levels of inflammatory markers among the three groups, including CRP, ferritin, lactate dehydrogenase (LDH), and D-dimer levels using certain cutoffs above which the levels were considered elevated. These cutoffs were designated as per institution protocol and was ≥ 100 mg/L for CRP, ≥ 530 ng/mL for ferritin, ≥ 590 U/L for LDH, and ≥ 0.5 mg/L for D-dimer respectively. We collected the baseline demographic data of the study population. Relevant clinical data associated with increased risk of MV, including a history of hypertension, diabetes mellitus, chronic liver disease, chronic pulmonary disease, coronary artery disease, and congestive heart failure, were collected. We also collected data regarding the different treatment modalities that each patient population received.

Ethical considerations: Institutional review board statement: The study was reviewed and approved by Saint Vincent-MetroWest Medical Center Institutional Review Board (approval No. 2020-035). Informed consent statement: The requirement of informed consent was waived by Saint Vincent- MetroWest Medical Center Institutional Review Board (approval No. 2020-035).

Data gathering and statistical analyses

The data was collected in Microsoft excel and was analyzed using SPSS. Non-parametric tests were employed since the data showed a non-normalcy distribution when we assessed it using the Shapiro-Wilk test. Chi-square analysis was employed for analyzing categorical variables and the Mann-Whitney U test was employed for analyzing continuous variables. Univariate logistic regression was utilized to assess the association between covariates and outcomes. We also calculated the area under the curve for invasive and NIMV for the different covariates, including CRP, ferritin, and LDH. The modalities of Medline, Pubmed and RCA were utilized to analyze high impact articles relevant to the current field of study and were incorporated in the discussion

RESULTS

Patient characteristics

Of the 290 patients screened, 118 met the inclusion criteria, among which 26 (22%) required IMV, 26 (22%) required NIMV, and 66 (56%) patients did not require any form of mechanical ventilatory support. There was an increased number of males in the group requiring IMV compared to those requiring NIMV (P = 0.01) (Table 1). Baseline demographics, including age > 60 years, vaccination status, and history of hypertension, diabetes mellitus, chronic liver disease, chronic pulmonary disease, coronary artery disease, and congestive heart failure, was similar among the three groups. In terms of medication administration, a significant difference was observed only in steroid use between patients on NIMV compared to those without (84.6% *vs* 66.7%, P = 0.01) (Table 1).

MV and inflammatory markers

The association between the levels of inflammatory markers and the use of invasive, non-invasive, and no mechanical ventilatory support was evaluated.

IMV: We observed a significant difference in the levels of inflammatory markers, including CRP (65.4% *vs* 25.8%, P = 0.01), ferritin (61.5% *vs* 36.4%, P = 0.01), troponin (42.3% *vs* 22.7%, P = 0.03), D-dimer (80.8% *vs* 51.5%, P = 0.01), and LDH (26.9% *vs* 4.5%, P = 0.04) between patients who required IMV and those who did not require MV (Table 2). This correlated with the significantly different mean levels of inflammatory markers observed between the two groups as well [CRP (160.2 *vs* 67, P = 0.001), ferritin (811 *vs* 295, P = 0.019), LDH (452 *vs* 321, P = 0.001) and D-dimer (2 *vs* 1, P = 0.001)]. Further univariate analysis between the inflammatory markers showed greater odds of having high inflammatory marker levels in patients who required IMV [CRP odds ratio (OR) 5.44, 95% confidence interval (CI): 2.04-14.48, ferritin (OR 2.8, 95%CI: 1.98-7.13), D-dimer (OR 3.95, 95%CI: 1.33-11.74), LDH (OR 7.73, 95%CI: 1.821-32.87), but troponin levels were not statistically significant (OR 2 .49, 95%CI: 0.947-6.56] (Table 3).

NIMV: A similar phenomenon of significantly different levels of inflammatory markers was observed in patients who required NIMV in comparison to those without mechanical ventilatory support requirements [CRP (53.8% *vs* 25.8%, P =

0.001), ferritin (65.4% *vs* 36.4%, *P* = 0.03), D-dimer (80.8% *vs* 51.5%, *P* = 0.01), and LDH (7.7% *vs* 4.5%, *P* = 0.001), but no significant difference was demonstrated in troponin levels (46.2% *vs* 22.7%, *P* = 0.06)] (Table 4). On assessing the mean levels of inflammatory markers between the two groups, we observed a significant difference in CRP (115.9 *vs* 67, *P* = 0.002), ferritin (628 *vs* 295, *P* = 0.013), and D-dimer (2 *vs* 1, *P* = 0.001) but no significant difference in LDH (357 *vs* 321, *P* = 0.29). We subjected these inflammatory biomarkers to univariate analysis, which showed increased odds of higher levels of all biomarkers except LDH among patients who required NIMV [CRP (OR 3.63, 95%CI: 1.30-8.67), ferritin (OR 3.306, 95%CI: 1.27-8.55), D-dimer (OR 3.95, 95%CI: 1.33-11.73), troponin (OR 2.94, 95%CI: 1.11-7.62) but no significant difference was demonstrated in LDH (OR 1.75, 95%CI: 0.27-11.12) (Table 5).

Area under curve-receiver operator characteristic (ROC) (AUROC): In order to further confirm the role of the inflammatory biomarkers in predicting the need for MV, ROC analysis was carried out. The AUROC for IMV was the following: for CRP, AUROC 0.747 (95%CI: 0.617-0.878, *P* = 0.001) that yielded a sensitivity of 80.8% and specificity of 50%; for ferritin, AUROC 0.658 (CI: 0.528-0.788, *P* = 0.019) with a sensitivity of 73% and specificity of 50%; for LDH, AUROC 0.699 (CI: 0.579-0.820, *P* = 0.003) with a sensitivity of 80.8% and specificity of 50%; and for D-dimer, AUC 0.751 (CI: 0.625-0.876, *P* = 0.001) with a sensitivity of 76.9% and specificity of 50% (Figure 1, Table 6).

The AUROC for NIMV was as follows: For CRP, AUROC 0.663 (95%CI: 0.527-0.799, *P* = 0.015) that yielded a sensitivity of 69.2% and specificity of 53%; for ferritin, AUROC 0.667 (CI: 0.555-0.778, *P* = 0.013) with a sensitivity of 80.8% and specificity of 53%; and for D-dimer, AUROC 0.740 (CI: 0.62-0.86, *P* = 0.004) with a sensitivity of 80.8% and specificity of 50% (Figure 2, Table 7).

DISCUSSION

This study is unique in assessing the utility of inflammatory markers, such as CRP, ferritin, LDH, and D-dimer in predicting the need for non-invasive as well as IMV in COVID-19 disease in patients with CKD. We observed that a higher proportion of COVID-19 patients with CKD who had elevated inflammatory marker levels ultimately required MV. The average inflammatory marker levels in all 3 groups (MV, NIMV and no MV) were high. Elevated levels of inflammatory markers were highly predictive of the need for IMV with corresponding AUROC of 0.747, 0.658, 0.699, and 0.751 for CRP, ferritin, LDH, and D-dimer, respectively. Although not all markers were predictive of the need for NIMV, CRP, ferritin, and D-dimer were predictive, with corresponding AUROCs of 0.663, 0.667, and 0.74, respectively. Although the pathophysiology explaining elevated LDH levels in patients requiring IMV but not amongst patients requiring NIMV is not explicitly clear, we hypothesize that this could be secondary to the LDH cutoff that was used to define levels as elevated. LDH enzyme plays a prominent role in active metabolism and levels are elevated with minor abnormalities such as tissue hypoxia and lysis necessitating a higher cutoff to detect significantly elevated LDH levels[8]. The results of our study reinforced the predictive value of CRP, ferritin, and D-dimer in patients with COVID-19 and underlying stages IIIb-V CKD. Among patients with CKD alone, studies have shown baseline elevated inflammatory marker levels, due to a chronic inflammatory milieu and decreased renal clearance of these inflammatory markers[7]. Our study highlighted the positive correlation of these markers with invasive as well as NIMV in COVID-19 patients with stages IIIb-V CKD; the high sensitivity of these markers demonstrated by the AUROC signifies their predictive potential.

In our study, the demographic variables were similar to the previous studies[4,5]. Male sex was associated with an increased risk of the need for invasive and NIMV. Sex may influence the severity of SARS-CoV-2 as the X-chromosome contains a higher density of immune-related genes and immunoregulatory elements related to innate and adaptive immunity[9]. There was an equal distribution of the need for MV in the presence of associated comorbidities, such as hypertension, diabetes mellitus, chronic liver disease, chronic obstructive pulmonary disease, coronary artery disease, and congestive heart failure. We noticed a significantly increased steroid administration rate in the NIMV group compared to the no MV group. One possible explanation for this finding could be the greater severity of the disease although there is no clear evidence to demonstrate the same

Biomarkers are a clinical reflection of the underlying disease process and help us assess the disease activity. This was frequently employed in COVID-19 disease with studies showing a correlation between elevated inflammatory marker levels and severe COVID-19 disease[5,6]. Although markers such as IL-6 were initially explored, they are cost-prohibitive and thus unsuitable for routine monitoring in COVID-19 patients[4]. This led to research on more routine biomarkers, including CRP, ferritin, LDH, and D-dimer, which have been shown to correlate well with the severity of COVID-19 disease[10]. Despite the use of different values of CRP to define elevation in multiple studies, such as Koozi *et al*[11] > 1000mg/L, Ryoo *et al*[12] > 140mg/L, and Liu *et al*[13] > 41.8 mg/L, there was a uniformly observed greater risk of severe COVID-19 disease[11-14].

Inflammatory markers are used for risk stratification and prognostication in several infectious diseases and malignancies, which are characterized by inflammation[15,16]. The pro-inflammatory nature of COVID-19 infection and associated organ dysfunction is well established[17,18]. Inflammatory markers, including CRP, erythrocyte sedimentation rate, LDH, and procalcitonin (PCT) are found to be elevated in patients with COVID-19[19,20]. Studies such as those by Herold *et al*[4] have demonstrated the utility of these biomarkers in prediction models that help detect the need for invasive and NIMV in patients with COVID-19 disease. They demonstrated an AUROC value of 0.97 and 0.86 for IL-6 and CRP with optimal cutoff values (IL-6: 80 pg/mL and CRP: 97 mg/L) that correctly classified 80% of their study population regarding their risk of respiratory failure[4]. The study by Li *et al*[5] used a multivariate stepwise logistic regression model to show the use of a glucocorticoid, increased neutrophil count, and PCT level in COVID-19 as predictive indicators for NIMV and the use of glucocorticoid increased neutrophil count and LDH level as effective predictors for

Table 1 Demographic information, *n* (%)

Variables	Invasive mechanical ventilation	Non-invasive mechanical ventilation	No mechanical ventilation	Total	*P* value[a]	*P* value[b]
Age > 60 yr	24	25	62	111	1.00	1.00
	92.3	96.2	93.9	94.1		
Male sex	18	12	26	56	0.01	0.55
	69.2	46.2	39.4	47.5		
Vaccinated against COVID-19	2	7	16	25	0.13	0.16
	7.7	26.9	24.2	21.2		
Hypertension	25	21	59	105	0.43	0.31
	96.2	80.8	89.4	89.0		
Diabetes mellitus	15	13	33	61	0.51	1.00
	57.7	50.0	50.0	51.7		
Chronic liver disease	0	1	1	2	1.00	0.49
	0.0	3.8	1.5	1.7		
Chronic obstructive pulmonary disease	8	11	13	32	0.26	0.26
	30.8	42.3	19.7	27.1		
Coronary artery disease	11	8	21	40	0.34	0.92
	42.3	30.8	31.8	33.9		
Congestive heart failure	10	11	17	38	0.23	0.12
	38.5	42.3	25.8	32.2		
Remdesivir	12	16	32	60	0.84	0.26
	46.2	61.5	48.5	50.8		
Steroids	24	22	44	90	0.01	0.12
	92.3	84.6	66.7	76.3		

[a]Chi square test between non mechanical ventilation and non invasive mechanical ventilation.
[b]Chi square test between non mechanical ventilation and invasive mechanical ventilation. COVID-19: Coronavirus disease 2019.

Table 2 Inflammatory marker levels between invasive mechanical ventilation and no mechanical ventilation, *n* (%)

Variables	Invasive mechanical ventilation	No mechanical ventilation	Total	*P* value[a]
CRP level (mg/L)	17	17	48	0.01
	65.4	25.8	40.7	
Ferritin level (ng/mL)	16	24	57	0.01
	61.5	36.4	48.3	
LDH level (U/L)	7	3	12	0.04
	26.9	4.5	10.2	
Troponin (ng/mL)	11	15	38	0.03
	42.3	22.7	32.2	
D-dimer (mg/L)	21	34	76	0.01
	80.8	51.5	64.4	

[a]Chi square test between non mechanical ventilation and non invasive mechanical ventilation. CRP: C reactive protein; LDH: Lactate dehydrogenase.

Table 3 Univariate analysis-invasive mechanical ventilation

Variables	OR	95%CI
CRP level	5.444	2.047-14.483
Ferritin level	2.8	1.098-7.138
LDH level	7.737	1.821-32.87
Troponin level	2.493	0.947-6.56
D-dimer level	3.953	1.331-11.74

CRP: C reactive protein; LDH: Lactate dehydrogenase; OR: Odds ratio; CI: Confidence interval.

Table 4 Inflammatory marker levels between non-invasive mechanical ventilation and no mechanical ventilation, *n* (%)

Variables	Non-invasive mechanical ventilation	No mechanical ventilation	Total	*P* value[a]
CRP level (mg/L)	14	17	48	0.001
	53.8	25.8	40.7	
Ferritin level (ng/mL)	17	24	57	0.03
	65.4	36.4	48.3	
LDH level (U/L)	2	3	12	0.001
	7.7	4.5	10.2	
Troponin (ng/mL)	12	15	38	0.06
	46.2	22.7	32.2	
D-dimer (mg/L)	21	34	76	0.01
	80.8	51.5	64.4	

[a]Chi square test between non mechanical ventilation and non invasive mechanical ventilation.
CRP: C reactive protein; LDH: Lactate dehydrogenase.

Table 5 Univariate analysis-non-invasive mechanical ventilation

Variables	OR	95%CI
CRP level	3.363	1.303-8.679
Ferritin level	3.306	1.277-8.55
LDH level	1.750	0.275-11.129
Troponin level	2.914	1.113-7.628
D-dimer level	3.953	1.331-11.736

CRP: C reactive protein; LDH: Lactate dehydrogenase; OR: Odds ratio; CI: Confidence interval.

IMV. In another single-center retrospective observational study, ferritin, LDH, absolute lymphocyte count, and CRP were found to predict the probability of early MIV with an accuracy of 88%[21].

The inflammatory markers are renally cleared, and hence reduced kidney function is associated with elevated levels of serum inflammatory markers. In addition, CKD is associated with chronic inflammation. Studies have demonstrated an elevation of CRP levels in patients with CKD and a negative correlation between CRP levels and glomerular filtration rate (GFR). There is evidence that inflammation, as measured by CRP level, increases with declining renal function in CKD patients[22-24]. A study by Keller *et al*[25] showed that in patients with initial stages of CKD and with end stage renal disease, the levels of CRP, fibrinogen, D-dimer, coagulation factor VII, factor VIII were increased, either due to increased production *vs* decreased clearance. CKD stages IIIb-V was selected since there was a significant increase in mortality rate amongst patients with CKD IIIb-V[26].

Table 6 Area under the curve-invasive mechanical ventilation

Variables on admission	AUC	*P* value	95% confidence interval		Sensitivity (%)	Specificity (%)
			Lower limit	Upper limit		
CRP level	0.747	0.001	0.617	0.878	80.8	51
Ferritin level	0.658	0.019	0.528	0.788	73	53
LDH level	0.699	0.003	0.579	0.820	80.8	51
D-dimer level	0.751	0.001	0.625	0.876	76.9	52

CRP: C reactive protein; LDH: Lactate dehydrogenase; AUC: Area under curve.

Table 7 Area under the curve-non invasive mechanical ventilation

Variables on admission	AUC	*P* value	95% confidence interval		Sensitivity (%)	Specificity (%)
			Lower limit	Upper limit		
CRP level	0.663	0.015	0.527	0.799	69.2	53
Ferritin level	0.667	0.013	0.555	0.778	80.8	53
LDH level	0.573	0.280	0.445	0.700	61.5	55
D-dimer level	0.740	0.0004	0.620	0.860	80.8	50

CRP: C reactive protein; LDH: Lactate dehydrogenase; AUC: Area under curve.

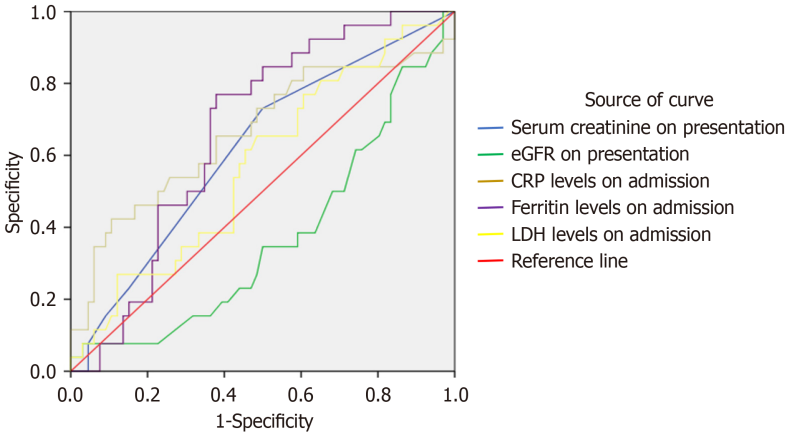

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Figure 1 Receiver operator characteristic curve for noninvasive mechanical ventilation. eGFR: Estimated glomerular filtration rate; CRP: C-reactive protein; LDH: Lactate dehydrogenase.

In our study, the mean CRP levels at admission in COVID-19 patients with stages IIIb-V CKD requiring IMV were remarkably higher than those who did not require MV (160.19 *vs* 67.02, $P = 0.001$). This finding likely reflects the impact of acute, severe COVID-19-related illness on the existing chronic inflammation in CKD, and concomitant reduced renal clearance of inflammatory markers. We found CRP, ferritin, LDH, and D-dimer to be good predictors of IMV and CRP, ferritin, and D-dimer to be good predictors of NIMV. Regardless of the negative correlation of inflammatory biomarkers with GFR in CKD, our study validated their high sensitivity in predicting COVID-19 prognosis in this specific population.

Limitations: One of the limitations of our study includes a small study population. We also did not include patients who had a history of renal transplantation, in order to minimize the influence of immunosuppressive medications in our study population. Another limiting factor includes the absence of information about baseline inflammatory marker levels in the setting of their underlying CKD. There are multiple factors that influence inflammatory marker levels, such as age, body mass index, sex, use of nicotine, blood pressure, and liver injury[20]. We did not study more specific markers such as IL-6, IL-1β, and IL-8, which are more sensitive but are cost-prohibitive in the real-world setting. We did not study the interactions of other comorbidities, interventions, and various medications with these inflammatory markers and the disease severity[27-29]. We also did not have the long term follow up details of these patients.

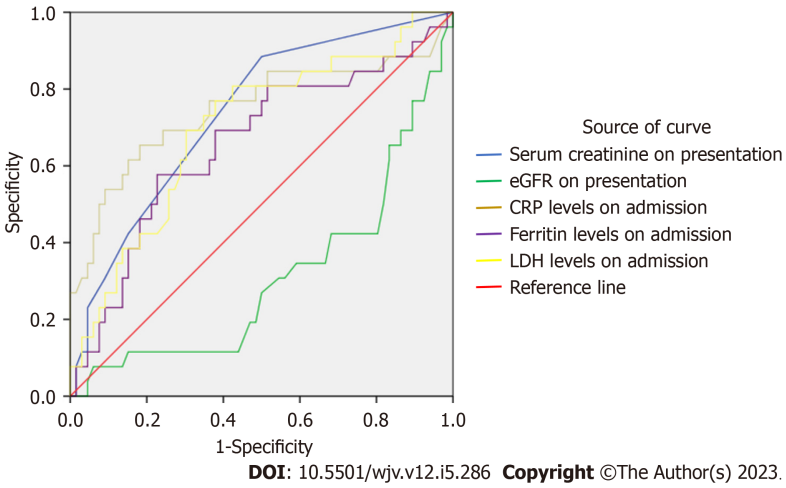

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Figure 2 Receiver operator characteristic curve for invasive mechanical ventilation. eGFR: Estimated glomerular filtration rate; CRP: C-reactive protein; LDH: Lactate dehydrogenase.

Future implications: Further prospective studies are needed to establish the correlation between the levels of inflammatory markers and the need for MV in COVID-19 patients with CKD. Validation of these inflammatory biomarkers is key in establishing their use as predictive indices. With the clinical utility of these inflammatory markers being described, it is imperative to study the impact of different disease processes on these inflammatory markers before employing them as clinical tools to guide the diagnosis and management of acute COVID-19 infection.

CONCLUSION

Our study explored the efficacy and predictive ability of inflammatory markers in detecting the risk of respiratory failure and the subsequent need for invasive and NIMV among COVID-19 patients with pre-existing CKD. We demonstrated that inflammatory markers, including CRP, ferritin, and D-dimer are useful predictive indicators of invasive and non-invasive MV in COVID-19 patients with stages IIIb-V CKD. The AUROC demonstrates good sensitivity for CRP levels in predicting the need for MV in the general population as well as in patients with stages IIIb-V CKD. This could be explained by the rationale that COVID-19 creates a greater magnitude of increased inflammation compared with increased inflammation due to CKD alone. With an increased need for better prognostic tools to help predict the severity of disease, especially among high-risk populations, and with the rising use of inflammatory markers to risk-stratify patients with COVID-19, large-scale, prospective studies are needed to delineate the optimal utilization of these biomarkers.

ARTICLE HIGHLIGHTS

Research background

Inflammatory markers have been validated in multiple studies to help predict the severity of disease and the need for mechanical ventilation (MV). Studies have shown baseline elevation in these same inflammatory markers in patients with chronic kidney disease (CKD) alone, due to a chronic inflammatory milieu in CKD and reduced renal clearance of these inflammatory markers. The clinical utility of these inflammatory markers to predict the need for MV among patients with coronavirus disease 2019 (COVID-19) and underlying CKD is unclear.

Research motivation

The use of biomarkers has been progressively increasing since the COVID-19 pandemic and the need for establishing the utility of these biomarkers in the presence of multiple comorbidities becomes essential to establish their clinical utility. Hence there is utmost need for this study to assess use of C-reactive protein level in assessing MV risk in CKD patients.

Research objectives

Since an increased level of inflammatory markers were observed in patients with chronic kidney disease, especially amongst those with stages IIIb-V, we planned to assess the utility of inflammatory biomarkers by evaluating the rate of MV and the levels of inflammatory biomarkers in stages IIIb-V chronic kidney disease patients who are diagnosed with COVID-19.

Research methods

In order to analyze the association between inflammatory marker levels and rate of MV, we did a single-center retrospective cohort study. The patients included in the study comprised of patients with stage IIIb-V CKD admitted to a community hospital with a diagnosis of COVID-19 infection. Amongst such patients, we extracted information regarding their inflammatory marker levels and their need for invasive and non-invasive MV (IMV) (NIMV) during their hospital stay.

Research results

A total of 290 patients were admitted between the study period of December 2019 to January, 2022 and amongst them 118 met the inclusion criteria. When we compared the rates of IMV, the group with IMV patients had a greater level of inflammatory markers. We also found a similar result when we compared the inflammatory marker levels amongst NIMV patients.

Research conclusions

Our results showed that elevated inflammatory marker levels were still associated with an increased rate of IMV and NIMV even amongst stage IIIb-V CKD patients with COVID-19 disease, thereby demonstrating the clinical utility of these biomarkers in assessing disease severity despite their baseline elevated levels observed in CKD patients.

Research perspectives

Validation of these inflammatory biomarkers is key in establishing their use as predictive indices. With the clinical utility of these inflammatory markers being described, it is imperative to study the impact of different disease processes on these inflammatory markers before employing them as clinical tools to guide the diagnosis and management of acute COVID-19 infection.

FOOTNOTES

Author contributions: Shanmugavel Geetha H and Martin S conceived the idea for the study; Shanmugavel Geetha H, GogtayM, Abraham GM, and Martin S designed and undertook the literature review; Shanmugavel Geetha H, Prabhu S, Sekar A, and Gogtay M collected data; Gogtay M and Singh Y performed the statistical analysis, figures, and appendix and analyzed and interpreted the data; Shanmugavel Geetha H, Prabhu S, Sekar A, Singh Y, and Gogtay M wrote the first draft of the manuscript; Shanmugavel Geetha H, Singh Y, Sekar A, Abraham GM, Martin S, Mishra AK and Gogtay M revised the subsequent drafts of the manuscript; all authors reviewed and agreed on the final draft of the manuscript.

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ORIGINAL ARTICLE

Basic Study

Perilipin2 inhibits the replication of hepatitis B virus deoxyribonucleic acid by regulating autophagy under high-fat conditions

Chuang Wang, Xiao-Yun Gao, Mei Han, Meng-Chun Jiang, Xiao-Yi Shi, Chun-Wen Pu, Xuan Du

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Abstract

BACKGROUND

Chronic hepatitis B virus (HBV) infection is often associated with increased lipid deposition in hepatocytes. However, when combined with non-alcoholic fatty liver disease or hyperlipidemia, it tends to have a lower HBV deoxyribonucleic acid (DNA) load. The relationship between lipid metabolism and HBV DNA replication and its underlying mechanisms are not well understood.

AIM

To investigate the relationship between lipid metabolism and HBV DNA replication and its underlying mechanisms.

METHODS

1603 HBsAg-seropositive patients were included in the study. We first explored the relationship between patients' lipid levels, hepatic steatosis, and HBV DNA load. Also, we constructed an HBV infection combined with a hepatic steatosis cell model *in vitro* by fatty acid stimulation of HepG2.2.15 cells to validate the effect of lipid metabolism on HBV DNA replication *in vitro*. By knocking down and overexpressing Plin2, we observed whether Plin2 regulates autophagy and HBV replication. By inhibiting both Plin2 and cellular autophagy under high lipid

stimulation, we examined whether the Plin2-autophagy pathway regulates HBV replication.

RESULTS

The results revealed that serum triglyceride levels, high-density lipoprotein levels, and hepatic steatosis ratio were significantly lower in the HBV-DNA high load group. Logistic regression analysis indicated that hepatic steatosis and serum triglyceride levels were negatively correlated with HBV-DNA load. Stratified analysis by HBeAg showed significant negative correlations between HBV-DNA load and hepatic steatosis ratio in both HBeAg-positive and HBeAg-negative groups. An *in vitro* cell model was developed by stimulating HepG2.2.15 cells with palmitic acid and oleic acid to study the relationship between HBV-DNA load and lipid metabolism. The results of the *in vitro* experiments suggested that fatty acid treatment increased lipid droplet deposition and decreased the expression of cell supernatant HBsAg, HBeAg, and HBV DNA load. Western blot and polymerase chain reaction analysis showed that fatty acid stimulation significantly induced Plin2 protein expression and inhibited the expression of hepatocyte autophagy proteins. Inhibition of Plin2 protein expression under fatty acid stimulation reversed the reduction in HBsAg and HBeAg expression and HBV DNA load induced by fatty acid stimulation and the inhibition of cellular autophagy. Knocking down Plin2 and blocking autophagy with 3-methyladenine (3-MA) inhibited HBV DNA replication.

CONCLUSION

In conclusion, lipid metabolism is a significant factor affecting HBV load in patients with HBV infection. The *in vitro* experiments established that fatty acid stimulation inhibits HBV replication *via* the Plin2-autophagy pathway.

Key Words: Lipid metabolism; Chronic HBV infection; Nonalcoholic fatty liver; Plin2; Autophagy

Core Tip: Our data suggest that fatty acid stimulation inhibits hepatitis B virus (HBV) replication by upregulating Plin2 expression, inhibiting hepatocyte autophagy. This process associates with lipid metabolism, autophagy pathway, and HBV replication. Further study of lipid metabolism-Plin2-autophagy is important to understand HBV host interactions and pathogenesis better and suggests a possible route for treating patients with chronic HBV infection combined with nonalcoholic fatty liver disease.

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INTRODUCTION

Hepatitis B virus (HBV) infection is a global health issue[1]. According to the World Health Organization, about one-third of the world's population will contract acute HBV at some point in their life[2]. Current main treatments include nucleoside analogs and interferon; however, they are not effective in eliminating the virus[3]. Therefore, it is imperative to better understand the underlying mechanisms behind HBV infection-induced disease to find new targets for anti-HBV therapy.

The relationship between HBV infection and lipid metabolism has received more attention in the last decade. Clinical studies have demonstrated that chronic HBV infection enhances the incidence of nonalcoholic fatty liver disease (NAFLD), with NAFLD co-infected with HBV accounting for 13.5% of all HBV patients[4]. These studies indicate a close association between HBV infection and altered lipid metabolism. This present study intends to clarify the mechanism of why increased lipid deposition in the liver can also inhibited HBV replication in hepatocytes[5].

Autophagy is an evolutionarily conserved catabolic process that regulates HBV replication and is required to maintain cellular homeostasis in response to the microenvironment. It involves selective and non-selective mechanisms that cause intracellular substrate degradation[6]. HBV was found to be able to maintain its own replication by inducing hepatocyte autophagy, and when cellular autophagy was inhibited, HBV replication expression in hepatocytes was significantly reduced[7]. Perilipin2 (Plin2) is involved in the formation of lipid droplets in the liver and peripheral tissues[8]. Plin2 is highly upregulated in humans and rodents with NAFLD[5,9]. Purposeful knockdown of Plin2 protein in the mouse liver was found to significantly reduce liver weight, body weight, and adipose tissue mass[10]. In a previous study on NAFLD pathogenesis, it was found that Plin2 is not only involved in intracellular lipid deposition but also regulates intracellular autophagy, and stimulation of hepatocytes with high concentrations of fatty acids results in increased Plin2 expression and cellular autophagy inhibition[11].

Combined with the above, we speculated that the Plin2-autophagy pathway might be involved in regulating lipid deposition and HBV replication in hepatocytes. In this study, we first explored the relationship between patients' lipid levels, hepatic steatosis, and HBV deoxyribonucleic acid (DNA) load. Also, we constructed an HBV infection combined with a hepatic steatosis cell model *in vitro* by fatty acid stimulation of HepG2.2.15 cells to validate the effect of lipid metabolism on HBV DNA replication *in vitro*. By knocking down and overexpressing Plin2, we observed whether Plin2 regulates autophagy and HBV replication. By inhibiting both Plin2 and cellular autophagy under high lipid stimulation, we examined whether the Plin2-autophagy pathway regulates HBV replication. This present study intends to investigate the relationship between lipid metabolism and HBV replication through retrospective analysis and *in vitro* studies, providing a novel theoretical basis for the mechanism of HBV replication and a new target for searching new therapeutic sites.

MATERIALS AND METHODS

Ethical statement

The study has been approved by the Ethics Committee of Dalian Sixth People's Hospital. The privacy rights of human subjects were always respected during human experimentation, and informed consent was obtained prior to the experiment. The ethics program number is DLY/CB-IRB-026.

Patient selection

In this cross-sectional hospital-based study, all patients were recruited from the Dalian Sixth People's Hospital. A total of 1603 HBsAg-positive patients underwent a comprehensive health examination with no prior antiviral treatments to evaluate the effect of lipid profile on HBV viral replication. Additionally, 132 chronic hepatitis B patients were included in the study to investigate the effect of antiviral treatment on lipid profile. Patients with hepatitis C and D, autoimmune hepatitis, alcoholic fatty liver, Wilson's disease, drug-related hepatic steatosis, liver surgery, or liver transplantation were excluded from the study.

Clinical data collection

During the study, data on age, sex, alcohol consumption, and medical history were collected through face-to-face interviews. Following an overnight fast, blood samples were obtained from all participants. HBsAg, antibodies against HBsAg, HBeAg, antibodies against HBeAg, and antibodies against hepatitis B core antigen were measured using an immunoassay analyzer. The levels of serum HBV-DNA copy were measured using the COBAS Amplicor HBV monitor test (Cap/ctm, Roche, Switzerland). Clinical chemistry systems were used to evaluate the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase, total bile acids, total bilirubin, albumin, and bile acid.

Reagents and antibodies

The HBV-producing HepG2.2.15 hepatoma cell line with an integrated HBV genomic dimer was obtained from the Chinese Academy of Sciences (CAS) in Beijing, China. Trypsin-EDTA (#27250–018), fetal bovine serum (#10100147), phosphate-buffered saline (PBS) (#226013), Opti MEM medium (#22600134), and Dulbecco's modified essential medium (DMEM) (#31600083) were purchased from GIBCO BRL (Grand Island, NY, United States). Oleic acid (OA, #15724), palmitic acid (PA, #27567713), and 3-methyladenine (3MA, #M9281) were acquired from Sigma-Aldrich (Missouri, United States). Perilipin2 (Plin2, #ac219686) was obtained from Abcam Technologies (Abcam, Cambridge, United Kingdom). Light chain 3 (LC3, #A11280), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, #AC002), anti-rabbit IgG (#AS014), and anti-mouse IgG (#LV-AS003) were obtained from Wuhan Abcotec Biotechnology Co Ltd (Abclonal, Wuhan, China).

Cell line, plasmid, and transfection

HepG2.2.15 cells were obtained from the CAS and cultured in T25 cell culture flasks with 10% heat-inactivated fetal bovine serum (GIBCO BRL) (#10100147), antibiotics, and high glucose DMEM (GIBCO) (#31600083). The flasks were pre-cultured at 37°C for 18-24 h in a 5% CO_2 incubator.

Plin2 knockdown and overexpression plasmids were synthesized by Suzhou Jima Bio. Plasmid transfection was performed following the manufacturer's instructions for Lipofectamine 2000 transfection reagent (Invitrogen).

Western blotting

Whole-cell protein extracts were obtained by passive cell lysis using protease and phosphorylated protease inhibitors following the manufacturer's instructions, and protein concentrations were determined using the BCA method. SDS-PAGE (10% gel) was used to separate samples containing approximately 30 μg of protein per well, and the proteins were transferred to PVDF membranes, which were incubated with primary antibody at 4°C overnight after being closed in low-fat milk powder for 2 h at room temperature in TBST. The membranes were washed and placed in BLOTTO containing secondary antibodies (HRP-labeled goat anti-rabbit antibody) for 1.5 h at room temperature. Following TBST clearing, the membranes were placed in the chromogen for 30 s and exposed immediately to the exposure cassette. The method was to estimate the ratio of the brightness value of each sample strip to the brightness value of the corresponding GAPDH (internal reference) strip to get the corrected strip brightness value.

Triglyceride content detection

The triglyceride content of HepG2.2.15 was measured using the Triglyceride Quantification Assay Kit (Abcam) for colorimetric detection, following the manufacturer's protocol.

Real-time quantitative polymerase chain reaction analysis of mRNA expression

Several methods have been described for the detection of HBV daughter DNA in culture supernatants[12,13]. Real-time reverse transcription (RT) polymerase chain reaction (PCR) assay using primers 5'TCTTGCCTTACTTTTGGAAG 3' (forward) 5'AGTTCTTCTTCTTCTAGGGGACC3' (reverse) were used to measure HBV pgRNA and Plin2 mRNA levels in cells.

Assays to detect HBsAg and HBeAg in the cell culture supernatant

HBsAg and HBeAg levels were evaluated using a commercial ELISA kit (Hunan Shengxiang Biotechnology Co., Ltd., Hunan, China) following the manufacturer's instructions. The absorbance of each well was sequentially measured at 450 nm wavelength with zero blank air conditioning for the final assay.

Oil red staining

After stimulating HepG2.2.15 cells with free fatty acids, the cells were rinsed three times with PBS and fixed in 10% formalin for 15 min at room temperature. Following fixation, the cells were stained with Oil Red O for 20 min at room temperature. Stained cells were observed by a fluorescent microscope (Leica DMI 4000 B) on the white light setting (magnification, ×100).

GFP-LC3 fluorescence analysis

After preincubation in a complete medium at 37°C in 21% O_2 and 5% CO_2 for 24 h, the cells were transfected with GFP-LC3 following the manufacturer's instructions to monitor autophagy flux. After 8 h of transfection, the cells were rinsed with PBS; a complete culture medium was added to the cells. Finally, the samples were observed under a fluorescence microscope (Nikon, Tokyo, Japan)

Statistical analyses

Continuous variables were reported as mean ± standard deviation or median (interquartile range). Dependent variables were expressed as numbers or percentages. The Wilcoxon matched-pairs signed-rank test, a nonparametric statistical test, was employed to compare non-normally distributed continuous data. This test was used to analyze various variables, including age, gender, FBG, ALT, AST, ALP, γ-GGT, LDH, bile acids, total cholesterol, triglycerides, HDL-C, LDL-C, apoA, and apoB. Chi-square test was used to compare the differences in hepatic steatosis prevalence and the HBeAg seropositive prevalence of patients with high or low HBV DNA load. Binary logistic regression analysis was conducted to identify potential factors influencing HBV DNA load, such as hepatic steatosis, triglycerides, apoA, apoB, cholesterol, HDL-C, and LDL-C. Statistical analysis was performed using IBM Corp's SPSS version 24.0 software. A two-sided P value < 0.05 was considered statistically significant. All experiments were conducted in triplicates, and western blot data were analyzed using t-tests.

RESULTS

Clinical characteristics of HBsAg-seropositive participants

In this study, 1603 HBsAg-seropositive patients were included, of which 674 (42.0%) were HBeAg-seropositive. Of the total patients, 1015 (63.3%) were male, and 815 (50.8%) had a high HBV viral load, defined as serum HBV DNA levels > 10^4 copies/mL. The median age was 52 years (range 43–60). Table 1 presents the characteristics of the HBsAg-seropositive patients. Patients in the high HBV DNA group had a higher levels of ALT, AST, ALP, γ-GGT, LDH, and HDL-C, and lower levels of triglyceride (TG), FBG, albumin, LDL-C, and apoB compared to those in the low HBV DNA group (P < 0.05). However, there were no significant differences between the two groups with regards to TC, total bilirubin, and apoA. The characteristics of the HBeAg-seropositive patients are shown in Table 2.

Clinical characteristics of HBeAg-seropositive and HBeAg-seronegative patients

Out of the 1603 HBsAg-seropositive patients, 661 were HBeAg-seropositive and 942 were HBeAg-seronegative. Among the HBeAg-seropositive group, 460 (69.6%) patients had a high viral load while in the HBeAg-seronegative group, 355 (37.7%) patients had a high viral load. Patients with high viral loads in the HBeAg-seropositive group had higher levels of ALT, AST, and HDL-C, and lower levels of albumin, FBG, LDL-C, apoB, and a lower ratio of steatosis (P < 0.05) (Table 1). In contrast, patients with high viral loads in the HBeAg-seronegative group had higher levels of ALT, AST, ALP, albumin, total bilirubin, and bile acid, and lower levels of TC, LDL-C, apoB, γ-GGT, TG, and a lower ratio of steatosis (P < 0.05) (Table 2).

Metabolic factors associated with HBV-DNA load in the HBsAg-seropositive participants

Table 3 presents the results of binary logistic regression analyses of metabolic factors associated with HBV-DNA load. The serum levels of TG (OR 0.83, 95%CI 0.70–0.98, P = 0.027), apoA (OR 0.47, 95%CI 0.26–0.83, P = 0.009), and LDL-C (OR

Table 1 Demographic, clinical, and laboratory characteristics of HBsAg-positive patients with high and low viral load using 10^4 copies per mL as the cutoff point, *n* (%)

Factors	ALL	High HBV DNA	Low HBV DNA	*P* value
	Case *n* = 1603	Case *n* = 815	Case *n* = 788	
Demographic				
Age (yr)	52 (43–60)	51 (40–60)	53 (45–61)	0.010
Male gender	1015 (63.28)	131 (69.6)	401 (68.2)	0.703
Laboratory tests				
FBG (mmol/L)	5.10 (4.61–5.87)	5.00 (4.5–5.7)	5.21 (4.77–6.00)	< 0.001
ALT (U/L)	47.95 (25.00–109.92)	68.3 (37.1–155.83)	31.7 (20.3–67.7)	< 0.001
AST (U/L)	41.19 (25.00–81.97)	52.95 (33.33–106.8)	29.86 (21.31–55.55)	< 0.001
ALP (U/L)	81.40 (63.63–109.00)	85.30 (66.00–116.60)	76.65 (61.9–104.00)	0.003
γ-GGT (U/L)	51.20 (22.89–113.36)	60.00 (29.13–135.00)	41.60 (18.97–100.12)	0.038
LDH (U/L)	194.8 (170.23–231.10)	196.00 (171.56–239.00)	194.00 (168.94–224.61)	0.006
Albumin (g/L)	40.70 (35.33–44.70)	39.60 (33.02–43.51)	41.96 (37.49–45.80)	0.045
Total bilirubin (μmol/L)	16.53 (11.63–25.24)	17.60 (12.20–27.70)	15.39 (11.37–23.20)	0.165
Bile acids (μg/mL)	11.00 (5.18–30.00)	14.30 (6.30–34.20)	8.70 (4.26–23.88)	0.002
Total cholesterol (mmol/L)	4.25 (3.55–5.01)	4.19 (3.50–4.92)	4.37 (3.61–5.10)	0.517
Triglycerides (mmol/L)	1.06 (0.73–1.53)	1.02 (0.73–1.44)	1.12 (0.74–1.71)	< 0.001
HDL-C (mmol/L)	1.15 (0.90–1.41)	1.19 (0.91–1.43)	1.12 (0.89–1.38)	0.029
LDL-C (mmol/L)	2.40 (1.87–2.98)	2.27 (1.76–2.89)	2.51 (1.95–3.10)	0.018
apoA (g/L)	1.19 (1.01–1.38)	1.14 (0.99–1.27)	1.18 (1.02–1.39)	0.849
apoB (g/L)	0.87 (0.70–1.06)	0.84 (0.69–1.01)	0.91 (0.73–1.10)	0.002
Steatosis	163 (10.2)	25 (3.1)	138 (17.5)	< 0.001
HBeAg sero-positive	661 (41.2)	460(56.4)	201 (25.5)	< 0.001

HBV: Chronic hepatitis B virus; FBG: Fasting blood glucose; ALT: Alanine Aminotransferase; AST: Aspartateaminotransferase; ALP: Alkaline Posphatase; LDH: lactic dehydrogenase; HDL: High density lipoprotein; LDL: Low- density lipoprotein; apo-A: Apolipoprotein-A; apo-B: Apolipoprotein-B.

0.59, 95%CI 0.45–0.77, $P < 0.001$) were negatively associated with HBV-DNA load, while hepatic steatosis (OR 0.15, 95%CI 0.10–0.23, $P < 0.001$) was also negatively associated with HBV-DNA load. On the other hand, TC level (OR 1.39, 95%CI 1.09–1.77, $P = 0.009$) was positively associated with HBV-DNA load.

Metabolic factors associated with HBV-DNA load in HBeAg-seropositive and HBeAg-seronegative participants

Table 3 provides the results of logistic regression analyses of metabolic factors associated with HBV-DNA load in HBeAg-seropositive and HBeAg-seronegative patients. In HBeAg-seropositive patients, serum LDL-C level (OR 0.38, 95%CI 0.24–0.60, $P < 0.001$) and hepatic steatosis (OR 0.11, 95%CI 0.05–0.22, $P < 0.001$) were negatively associated with HBV-DNA load, while TC level (OR 2.33, 95%CI 1.55–3.51, $P < 0.001$) was positively associated with HBV-DNA load. In contrast, in HBeAg-seronegative patients, serum TG level (OR 0.69, 95%CI 0.55–0.87, $P = 0.002$), LDL-C level (OR 0.46, 95%CI 0.31–0.67, $P < 0.001$), and hepatic steatosis (OR 0.19, 95%CI 0.11–0.35, $P < 0.001$) were negatively associated with HBV-DNA load, while TC level (OR 1.69, 95%CI 1.19–2.40, $P = 0.004$) was positively associated with HBV-DNA load.

In vitro high-fat conditions inhibit the replication of HBV DNA

To investigate the relationship between lipid metabolism and HBV DNA replication *in vitro*, HepG2.2.15 cells were stimulated with varying concentrations of PA and oleic OA to create a model of HBV infection combined with hepatic steatosis. Real-time PCR was used to detect changes in HBV DNA replication levels. The results demonstrated that the expression load of HBV DNA significantly decreased in a concentration-dependent manner with the increase of PA or OA concentration (Figure 1A and B). The optimal stimulation concentration of OA was 0.2 M, while the optimal stimulation concentration of PA was 100 μmol/L. The optimal fatty acid concentrations were prepared into free fatty acids (FFA) at an OA:PA ratio of 2:1. After 72 h of FFA treatment, HepG2.2.15 cells were stained with oil red O hematoxylin, revealing a significant increase in intracellular lipid droplets in the FFA group compared to the control group, and fusion phenomena were observed (Figure 1C). The intracellular TG content was higher in the high-fat

Table 2 Clinical characteristics of HBeAg-seropositive and HBeAg-seronegative patients with high and low viral load using 10^4 copies per mL as the cutoff point, *n* (%)

Factors	HBeAg sero-positive			HBeAg sero-negative		
	High HBV DNA	Low HBV DNA	*P* value	High HBV DNA	Low HBV DNA	*P* value
	Case *n* = 460	Case *n* = 201		Case *n* = 355	Case *n* = 587	
Demographic						
Age (yr)	45 (36–59)	50 (40–59)	0.106	54 (45–61)	55 (46–61)	0.564
Male gender	314 (68.2)	137 (68.1)	0.412	128 (56.6)	93 (68.4)	0.026
Laboratory tests						
FBG (mmol/L)	4.83 (4.40–5.35)	5.10 (4.60–5.90)	< 0.001	5.23 (4.73–6.20)	5.28 (4.80–6.06)	0.795
ALT (U/L)	83.21 (43.45–190.62)	51.60 (26.62–120.07)	< 0.001	53.00 (3200–127.00)	27.60 (19.38–53.60)	< 0.001
AST (U/L)	59.30 (38.01–130.1)	46.85 (26.00–93.18)	< 0.001	46.50 (37.57–116.55)	27.00 (20.72–46.47)	< 0.001
ALP (U/L)	87.50 (67.98–117.09)	90.18 (67.00–127.15)	0.489	83.40 (65.20–111.90)	74.00 (60.00–96.00)	< 0.001
γ-GGT (U/L)	64.61 (33.50–139.92)	68.25 (33.25–145.87)	0.615	52.00 (21.90–115.69)	69.6 (33.40–164.30)	< 0.001
LDH (U/L)	195.00 (171.92–238.32)	193.40 (167.37–228.97)	0.38	197.00 (170.08–239.14)	194.00 (169.00–222.39)	0.077
Albumin (g/L)	39.40 (32.81–43.36)	40.30 (34.89–44.50)	0.026	54.00 (45.00–61.50)	43.90 (38.00–47.70)	< 0.001
Total bilirubin (μmol/L)	17.90 (12.40–28.35)	17.80 (13.21–32.52)	0.751	17.20 (11.9–27.00)	14.71 (11.08–22.47)	< 0.001
Bile acids (ug/mL)	16.60 (8.01—38.77)	16.50 (8.01–41.52)	0.904	10.06 (4.98–27.50)	7.07 (3.73–16.54)	< 0.001
Total cholesterol (mmol/L)	4.16 (3.50–4.85)	4.00 (3.30–4.83)	0.674	4.22 (3.49–5.01)	4.46 (3.71–5.15)	0.019
Triglycerides (mmol/L)	1.03 (0.73–1.44)	1.08 (0.71–1.71)	0.429	1.04 (0.73–1.41)	1.14 (0.75–1.72)	0.002
HDL-C (mmol/L)	1.19 (0.90–1.43)	1.08 (0.81–1.40)	0.041	1.20 (0.92–1.44)	1.12 (0.91–1.38)	0.13
LDL-C (mmol/L)	2.25 (1.76–2.90)	2.41 (1.89–3.06)	0.041	2.31 (1.72–2.87)	2.54 (1.97–3.12)	< 0.001
apoA (g/L)	1.17 (0.97–1.35)	1.15 (0.92–1.30)	0.204	1.21 (1.00–1.40)	1.20 (1.04–1.41)	0.624
apoB (g/L)	0.85 (0.69–1.02)	0.91 (0.70–1.12)	0.01	0.83 (0.68–1.00)	0.91 (0.74–1.09)	< 0.001
Steatosis	11 (2.4)	37 (18.4)	< 0.001	11 (3.0%)	101 (17.2)	< 0.001

HBV: Chronic hepatitis B virus; FBG: Fasting blood glucose; ALT: Alanine Aminotransferase; AST: Aspartateaminotransferase; ALP: Alkaline Posphatase; LDH: Lactic dehydrogenase; HDL: High density lipoprotein; LDL: Low- density lipoprotein; apo-A: Apolipoprotein-A; apo-B: Apolipoprotein-B.

stimulation conditions than in the control group (Figure 1D). ELISA was used to measure HBsAg and HBeAg levels in cell culture supernatants, and it was found that high-fat stimulation inhibited the expression of both HBsAg (Figure 1E) and HBeAg (Figure 1F).

Based on these findings, it can be concluded that high-fat conditions inhibit the expression of HBV DNA and its serum markers in a concentration-dependent manner. However, the exact mechanism underlying this inhibition remains unclear.

Fatty acids stimulation promoted the expression of Plin2 and inhibited the replication of HBV DNA

To investigate the role of Plin2 in inhibiting HBV replication under high lipid conditions, we performed both Plin2 knockdown and overexpression experiments in HepG2.2.15 cells after fatty acid stimulation. After downregulating Plin2 protein, the number and volume of intracellular lipid droplets significantly decreased in both the control group and FFA-stimulated group under microscopy, whereas the number of lipid droplets increased in the Plin2 overexpression group (Figure 2A). Additionally, TG content was observed to increase in the Plin2 overexpression group (Figure 2B and C). HBV DNA load increased significantly after knockdown of Plin2 (Figure 2D-F). The expression of HBsAg and HBeAg was also significantly upregulated after transfection with siPlin2 plasmid, whereas the expression of both markers was downregulated after overexpression of Plin2 (Figure 2G-J).

These findings suggest that high lipid conditions upregulate Plin2 expression and that Plin2 plays a role in counteracting the regulation of lipid metabolism.

Plin2 affects HBV DNA replication by regulating autophagy

Western blotting was used to examine the expression of Plin2 and autophagy-related proteins LC3-II and LC3-I. The

Table 3 Results of univariate and multivariate analyses on lipid level and steatosis and chronic hepatitis B viru deoxyribonucleic acid load

	P value	OR (95%CI)
HBsAg sero-positive		
Steatosis	< 0.001	0.15 (0.10–0.23)
Triglyceride	0.027	0.83 (0.70–0.98)
apoA	0.009	0.47 (0.26–0.83)
apoB	0.905	0.99 (0.91–1.08)
Cholesterol	0.009	1.39 (1.09–1.77)
HDL-C	0.171	1.38 (0.87–2.18)
LDL-C	< 0.001	0.59 (0.45–0.77)
HBeAg sero-positive		
Steatosis	< 0.001	0.11 (0.05–0.22)
Triglyceride	0.077	0.74 (0.53–1.03)
apoA	0.846	1.10 (0.41–2.96)
apoB	0.5	0.69 (0.23–2.02)
Cholesterol	< 0.001	2.33 (1.55–3.51)
HDL-C	0.204	0.61 (0.29–1.30)
LDL-C	< 0.001	0.38 (0.24–0.60)
HBeAg sero-negative		
Steatosis	< 0.001	0.19 (0.11–0.35)
Triglyceride	0.002	0.69 (0.55–0.87)
apoA	0.209	0.62 (0.29–1.31)
apoB	0.713	1.02 (0.91–1.14)
Cholesterol	0.004	1.69 (1.19–2.40)
HDL-C	0.904	0.96 (0.51–1.81)
LDL-C	< 0.001	0.46 (0.31–0.67)

HBV: Chronic hepatitis B virus; FBG: Fasting blood glucose; ALT: Alanine Aminotransferase; AST: Aspartateaminotransferase; ALP: Alkaline Posphatase; LDH: Lactic dehydrogenase; HDL: High density lipoprotein; LDL: Low- density lipoprotein; apo-A: Apolipoprotein-A; apo-B: Apolipoprotein-B.

results showed that Plin2 expression was significantly upregulated under fatty acid stimulation conditions, and Plin2 knockdown under fatty acid stimulation conditions demonstrated a decreasing trend in upregulated Plin2 expression (Figure 3A-C). Autophagy-related proteins LC3-II/LC3-I were significantly decreased when HepG2.2.15 cells were stimulated with fatty acids, indicating autophagy inhibition. Autophagy expression increased when Plin2 expression was disturbed; after knockdown of Plin2 under high-fat conditions, autophagy was restored, whereas overexpression of Plin2 under both normal medium and high-fat stimulation significantly inhibited autophagy (Figure 3B and D). The number of autophagic vesicles significantly decreased when HepG2.2.15 cells were stimulated with fatty acids, indicating that autophagy was inhibited as seen through GFP-LC3 staining. The number of autophagic vesicles increased when Plin2 expression was interfered with and significantly decreased when Plin2 expression was overexpressed (Figure 3E).

Based on these findings, it can be concluded that fatty acid stimulation alters the autophagic trend by affecting the expression of Plin2, thereby affecting HBV DNA replication.

Lipid metabolism affects HBV DNA replication through Plin2autophagy-related pathway

Plin2 knockdown under fatty acid stimulation was observed to restore the inhibited HBV replication, whereas the restored DNA expression load was again inhibited after adding 3-MA to inhibit autophagy (Figure 3F). Plin2 protein expression remained unchanged upon the addition of autophagy inhibitor, as observed through Western blot analysis (Figure 3G and H). In contrast, the values of autophagy-related protein LC3-II/LC3-I were significantly downregulated (Figure 3G and I), indicating that autophagy was clearly inhibited. These results suggest that fatty acid stimulation inhibits autophagy and HBV DNA replication *via* Plin2.

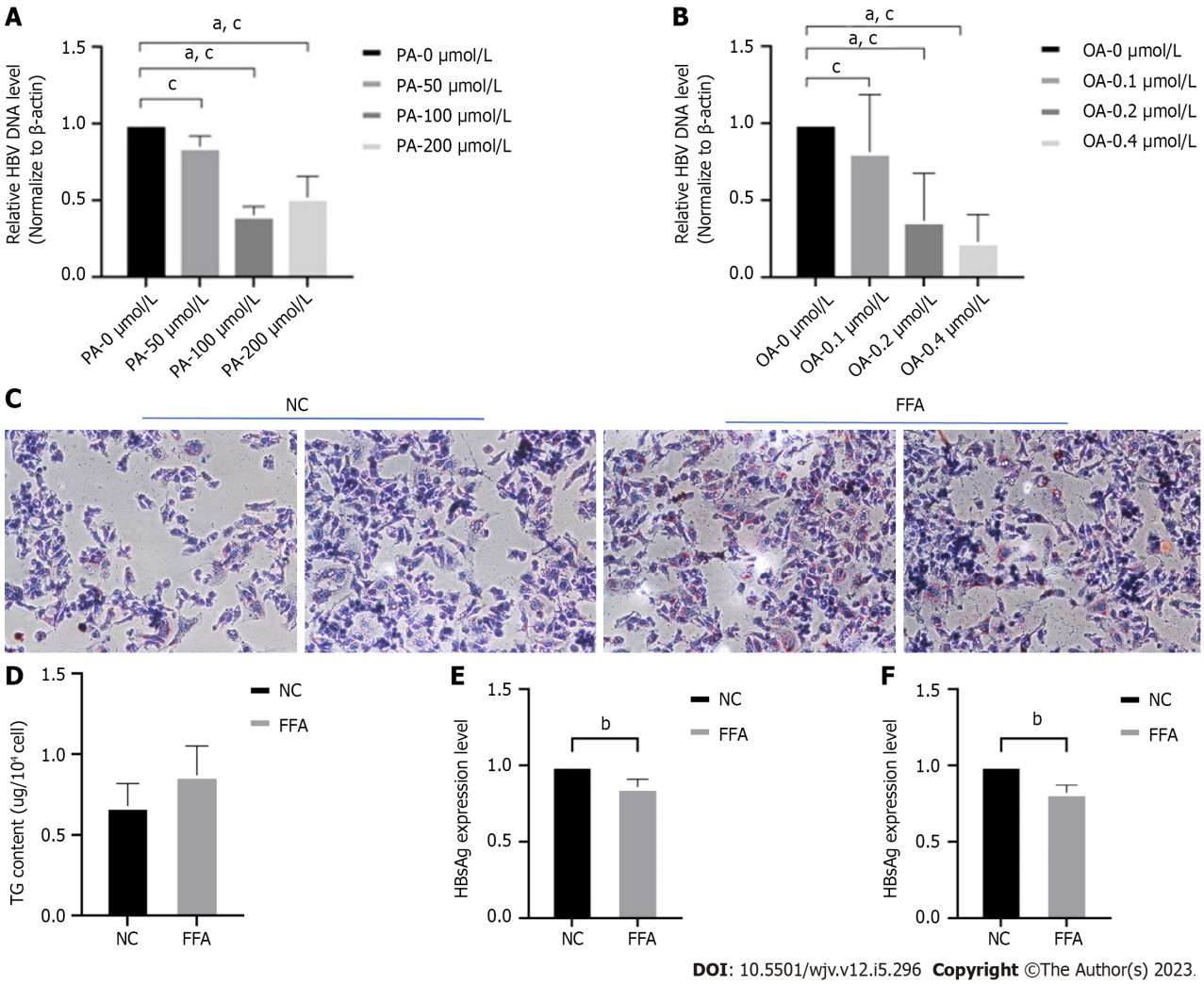

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Figure 1 *In vitro*, high lipid cases promote lipid droplet formation and inhibit chronic hepatitis B virus deoxyribonucleic acid replication and the choreographing of related antibodies. A and B: HepG2.2.15 cells were stimulated with different concentrations of palmitic acid (PA) and oleic acid (OA) for 48 h, and the expression of chronic hepatitis B virus deoxyribonucleic acid was detected; C: 0.2 mmol/L concentration of OA and 100 μmol/L concentration of PA were applied to stimulate HepG2.2.15 cells for 48 h, and the intracellular lipid droplet formation was detected by applying oil red O staining method; D: Detection of intracellular triglyceride content in both groups; E and F: After applying free fatty acids stimulation for 48 h, the levels of HBsAg and HBeAg secreted by the two groups of cells were detected by the ELISA method, respectively. [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$. NC: Nucleocapsid protein; FFA: Free fatty acids.

DISCUSSION

HBV, a DNA virus that causes immune-mediated liver disease, can be transmitted through blood and body fluids. Although immunomodulatory drugs such as interferon and antiviral drugs have a good safety profile and are also effective in controlling HBV replication in patients with chronic hepatitis B, they rarely eliminate HBV completely and do not completely eliminate liver cancer risk[14]. Therefore, there is an urgent requirement to explore the deeper regulatory mechanisms of HBV in order to seek the development of new drugs for HBV clearance.

Hepatic steatosis is characterized by an excessive accumulation of triglycerides in hepatocytes[15]. In previous studies, hepatic steatosis has been found in patients with chronic HBV infection, causing a reduction in their response to antiviral therapy[16]. However, there is mixed evidence on the association between hepatic steatosis and HBV replication. Several studies have shown that hepatic steatosis induces HBsAg clearance and reduces HBV replication[17,18]. Chia-Ming Chu's study showed that in patients with increased body mass index, hepatic steatosis accelerated HBsAg serological clearance by approximately 5 years[19]. Conversely, Lesmana *et al*[20] found no difference in HBV replication between HBV patients with and without hepatic steatosis. Our results indicated that serum triglycerides, HDL levels, and the rate of hepatic steatosis were significantly lower in the HBV-DNA high load (815 cases) group compared to the HBV-DNA low load (788 cases) group. The findings of logistic regression demonstrated that serum TG levels (OR 0.83 95%CI 0.70–0.98, $P = 0.027$), apoA levels (OR 0.47, 95%CI 0.26–0.83, $P = 0.009$), LDL-C levels (OR 0.59, 95%CI 0.45–0.77, $P < 0.001$) and hepatic steatosis (OR 0.15, 95%CI 0.10–0.23, $P < 0.001$) showed significant negative correlation with HBV-DNA load. As a result, we conducted a stratified analysis according to HBeAg serostatus. The results of logistic regression showed that hepatic steatosis serum triglyceride load was negatively correlated with blood HBV-DNA load in both HBeAg positive or negative groups ($P < 0.001$). These findings are consistent with a study reported by Jarcuska *et al*[21], which stated a

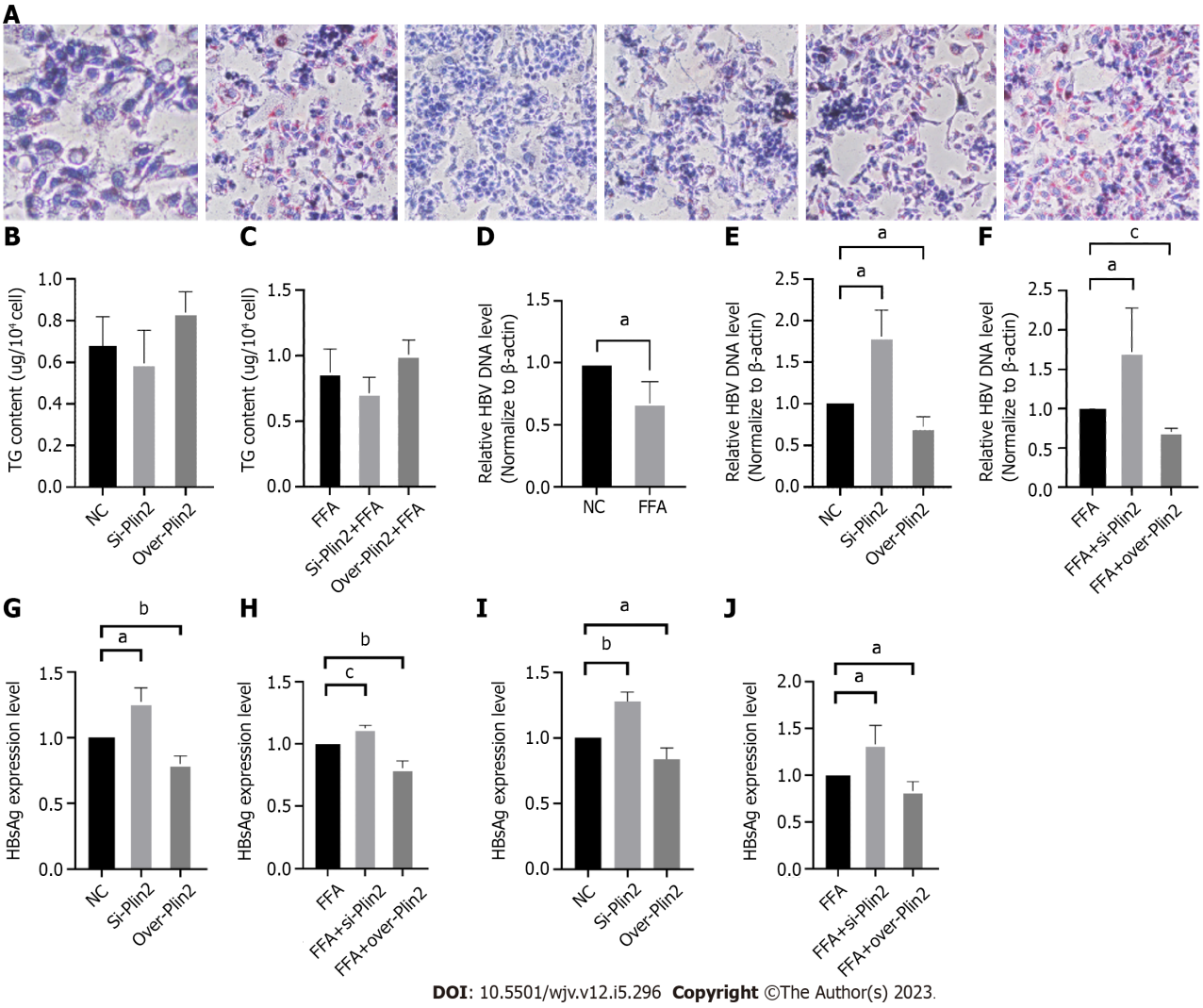

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Figure 2 To investigate the role of Plin2 in the effect of high-lipid conditions on chronic hepatitis B virus. A: Oil red O staining to observe lipid droplet formation; B and C: Triglyceride (TG) assay kit was applied to detect the TG content of each group; D-F: Quantitative polymerase chain reaction method was applied to detect the chronic hepatitis B virus deoxyribonucleic acid content in each group; G-J: HBsAg and HBeAg levels were detected in each group by the ELISA method. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.

significantly lower HBV-DNA load in patients with hypertriglyceridemia. These findings indicate that increased lipid metabolism in the body can inhibit HBV replication.

Previous studies have demonstrated that autophagy is closely associated with HBV DNA replication, and various factors in HBV infection, such as interferon Alpha and endoplasmic reticulum stress, can affect HBV replication by inducing autophagy[22]. Yongjun Tian *et al*[23] found that after liver-specific Atg5 knockdown in the HBV Tg05 mouse, the serum levels of HBeAg and HBsAg were decreased by about 50% and 60%, respectively. However, this autophagy inhibition decreased HBV DNA levels by more than 90%. A previous study showed that IFNα-2a treatment promoted autophagy initiation and blocked autophagy degradation, leading to a slight enhancement of HBV replication[24]. Autophagy disorders often result in metabolic abnormalities and play an essential role in the pathogenesis of numerous metabolic liver diseases, such as alcoholic liver disease and NAFLD[25]. Singh *et al*[26] coined the term "lipophagy" after identifying autophagy-mediated lipolytic functions in the LIPA pathway; TSAI T H showed that specific knockdown of Plin2 decreased triglyceride levels in mice by approximately 60%[27]. In another study of liver-specific Plin2 knockout mice, a significant increase in LC3 and p62-positive spots were detected in the livers of Plin2-deficient mice fed WTD[28]. In the study by Tsai *et al*[27], it was found that the down-regulation of Plin2 stimulated TG catabolism by upregulating autophagy expression through direct knockdown of Plin2. Therefore, we speculated that the high-fat environment regulates the Plin2-autophagy pathway and thus inhibits HBV DNA replication. Our experiments first examined the relationship between Plin2 and autophagy. Autophagy expression increased both under normal medium and under high-fat stimulation when Plin2 protein expression was down-regulated, and overexpression of Plin2 protein resulted in significant inhibition of autophagy. Fluorescence microscopy showed an increase in autophagy vesicles when Plin2 protein expression was down-regulated. Then the association between Plin2 and autophagy under fatty acid stimulation was further explored. Plin2 expression was significantly upregulated in HepG2.2.15 cells, and cellular autophagy was significantly inhibited. Compared to the control group, the expression of supernatant HBV DNA load and serological

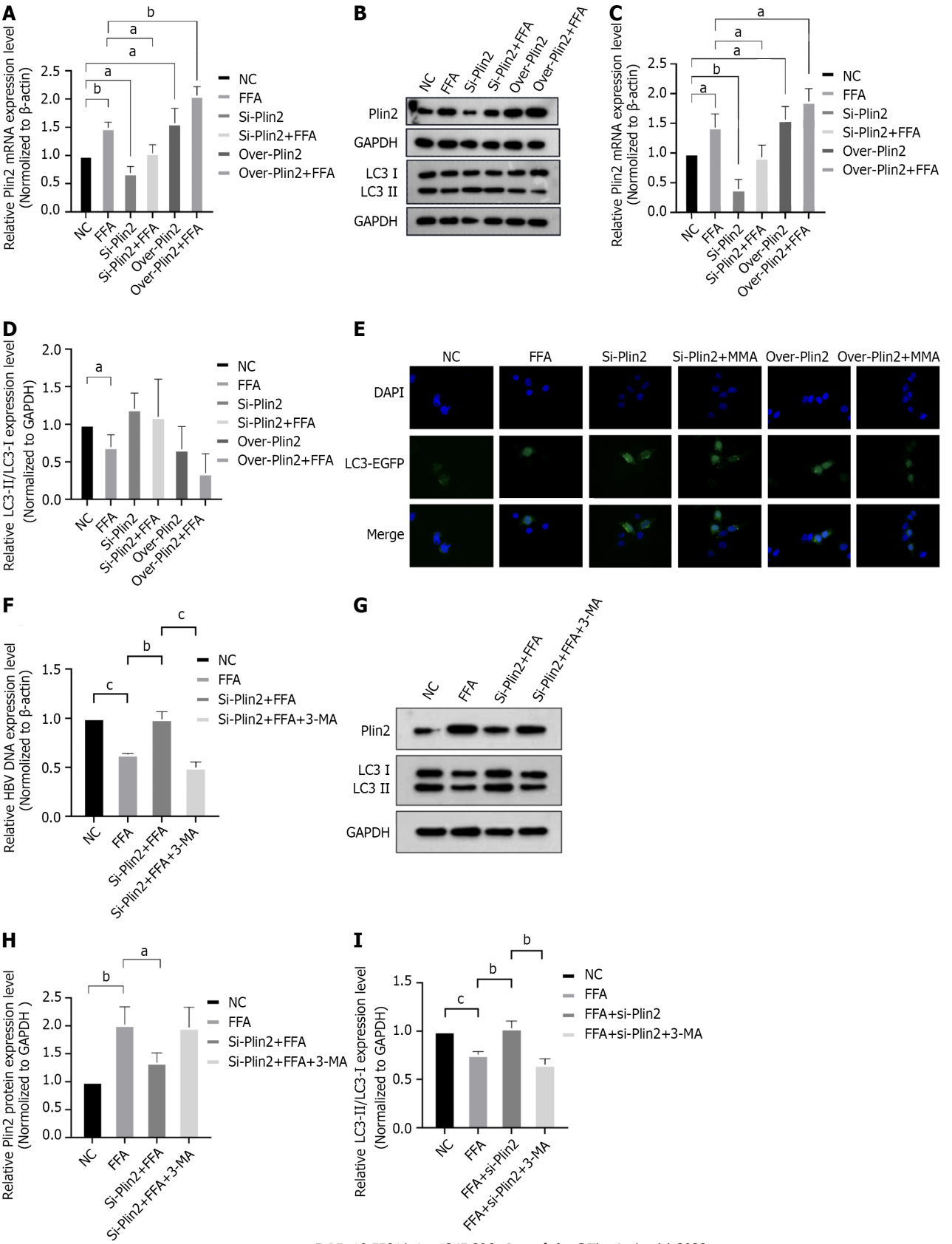

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Figure 3 Abnormal lipid metabolism affects chronic hepatitis B virus deoxyribonucleic acid replication through the Plin2-autophagy-related pathway. A: Quantitative polymerase chain reaction (q-PCR) method was applied to detect Plin2 mRNA levels; B-D: Western blot to detect the expression of Plin2 and LC3 in each group and detect the grayscale value; E: GFP-LC3 formation was observed under the fluorescence microscope; F: Chronic hepatitis B virus deoxyribonucleic acid levels were detected by applying q-PCR; G-I: Western blot was performed to detect the expression of Plin2 and LC3 in each group and to detect the grayscale values. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.

markers in HepG2.2.15 cells were significantly lower in the Plin2 knockdown group, whereas the overexpression of Plin2 was reversed. Finally, we inhibited the growth of autophagy with 3-MA in parallel with the knockdown of Plin2. At that time, we found that the growth of autophagy-related proteins was significantly reduced. In addition, the increased HBV DNA replication was also reduced, and HBsAg and HBeAg were similarly altered. All these results indicate that the Plin2-autophagy pathway is involved in the regulation of high-fat inhibition of HBV replication.

CONCLUSION

In summary, our data suggest that fatty acid stimulation inhibits HBV replication by upregulating Plin2 expression, inhibiting hepatocyte autophagy. This process associates with lipid metabolism, autophagy pathway, and HBV replication. Further study of lipid metabolism-Plin2-autophagy is important to understand HBV host interactions and pathogenesis better and suggests a possible route for treating patients with chronic HBV infection combined with NAFLD.

ARTICLE HIGHLIGHTS

Research background

The relationship between lipid metabolism and hepatitis B virus (HBV) deoxyribonucleic acid (DNA) replication and its underlying mechanisms are not well understood.

Research motivation

To investigate the relationship between lipid metabolism and HBV DNA replication and its underlying mechanisms.

Research objectives

We speculated that the Plin2-autophagy pathway might be involved in regulating lipid deposition and HBV replication in hepatocytes.

Research methods

We first explored the relationship between patients' lipid levels and HBV DNA load. Also, we constructed an HBV infection combined with a hepatic steatosis cell model *in vitro*.

Research results

Stratified analysis by HBeAg showed significant negative correlations between HBV-DNA load and hepatic steatosis ratio in both HBeAg-positive group and in HBeAg-negative group. The results of *in vitro* experiments suggested that fatty acid treatment increased the lipid droplets deposition and decreased the cell supernatant HBsAg, HBeAg expression and HBV DNA load.

Research conclusions

Fatty acid stimulation inhibits HBV replication by upregulating Plin2 expression, inhibiting hepatocyte autophagy.

Research perspectives

A possible route for treating patients with chronic HBV infection combined with nonalcoholic fatty liver disease.

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FOOTNOTES

Co-first authors: Chuang Wang and Xiao-Yun Gao.

Co-corresponding authors: Chun-Wen Pu and Xuan Du.

Author contributions: Du X and Gao XY designed the experiment and drafted the manuscript; Wang C performed the experiments; Du X and Wang C participated in the statistical analyses; Han M, Shi XY, and Jiang CM helped draft the manuscript; All authors have read and approved the final manuscript.

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