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Insight into the liver dysfunction in COVID-19 patients: Molecular mechanisms and possible therapeutic strategies

Khullar N *et al*. Mechanisms of liver damage in COVID-19 patients

Abstract

The terror of coronavirus disease 2019 (COVID-19) shrouded the entire world and captured more than 530 million people till June 2022. Though it is popularly famous for its despotic respiratory distress (Severe acute respiratory syndrome), meta-analysis has resonated an alert signal of liver dysfunction towards patients meeting severe symptoms. Current understanding reveals peculiar patterning in the receptors on the hepatic cells aiding viral invasion (expression of angiotensin converting enzyme receptors); anomalous liver biochemistry [lactate dehydrogenase, Ferritin, interleukin (IL)-2, IL-6, alanine aminotransferase/aspartate aminotransferase, bilirubin, *etc.*]; and thwarting effects on patients with existing liver diseases. It has also been expounded that in some cases, Covid pharma therapy (Mitifovir, Lopinavir/Ritonavir, Tocilizumab, *etc.*) triggered liver injury and cholestatic toxicity. Several proven indicators buttress cytokine storm-induced hepatic damage. The global figure of 1.5 billion chronic liver disease patients is further petrifying. Thus, it becomes imperative to critically evaluate the molecular mechanisms concerning hepatotropism of COVID-19, which could usher some new outlines of potential therapeutics. This review also designates a comprehensive outlook showing comorbidity, lifestyle impact, and genetics in the treatment management of COVID-19 patients.

Key Words: COVID-19; Liver damage; Pharmacotherapy; Cytokines storm; Molecular mechanisms

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Core Tip: Several review articles in the literature contributed to the pathophysiology, therapeutic strategies, vaccine development, and clinical trials for coronavirus disease 2019 (COVID-19) pathophysiology. Since liver is the primary site of synthesis of

proteins associated with immunity, these barricades the infectious microbial infestation inflowing from the gut into the bloodstream. Any liver defect would thus cause a compromised immune system. Patients with chronic liver disease were the worst hit since COVID-19 pandemic. This review article demonstrated the pathophysiology and molecular mechanisms responsible for distraught in the immune system that calls for more severe outcomes in patients with hepatic defects. Further, we critically evaluate the molecular mechanisms concerning hepatotropism in COVID-19 patients, which could usher some new outlines of potential therapeutics.

INTRODUCTION

Everything came to a standstill as the world witnessed a strange contagion on December 31, 2019, that surfaced enigmatic reports of patients with the unusual severe acute respiratory syndrome (pneumonia) in Wuhan, China. On January 7, 2020, the causal agent responsible for the mysterious deaths was branded as a novel coronavirus (2019-nCoV) by the International Classification Committee of Viruses[1]. It was avowed as a severe global pandemic on March 11, 2020, and since then, there have been 623893894 confirmed cases of coronavirus disease 2019 (COVID-19), including 6553936 deaths, as of October 21, 2022[2]. The clinical manifestation of COVID-19 is usually interpreted as severe lung infection (acute respiratory distress syndrome), causing turmoil in the patient's respiratory system and even death[3]. Many individuals admitted to intensive care units were known to have hepatic and heart-related complications[4]. Patients with chronic liver disease (CLD) were the worst hit since both severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and CLD hamper the immune system leading to an elevated and dysregulated immune response[5]. Not only this, but even healthy individuals infected with COVID-19 displayed a despising liver function, implicating a grander intensification in morbidity and mortality of such patients[6]. Since then, hepatologists have been working to deduce the relationship between COVID-19 and the liver. About 300 million CLD patients in China alone have been found to face the vilest effect of COVID-19[7]. It is advocated that SARS-CoV-2

invasion might be having a triggering and snowballing reactivation effect on the standing liver disorders in the patient, causing hepatotoxicity[8]. Also, hepatic patients are more prone to COVID-19[9]. Some cases have reported massive cytokine upsurge owing to multiorgan failure, including vascular endothelial growth factor, interleukin (IL)-6, macrophage inflammatory protein 1α, macrophage inflammatory protein 1β exudation during COVID-19[9-11]. Since the liver is a primary site of the synthesis of proteins associated with immunity, it barricades the infectious microbial infestation inflow from the gut into the bloodstream. Any liver defect would thus cause a compromised immune system[12]. Also increased level of hepatic enzymes [alanine transaminase (ALT) and aspartate transaminase (AST)] is a clear biomarker indicating the close pathophysiological association between the liver and COVID-19. Obesity further disposes individuals more susceptible to metabolic disorders, diabetes, and insulin resistance and by the means causing CLD, which may culminate into cirrhosis, fibrosis, and even hepatocellular carcinoma[13]. This indicates a dire need to investigate the pathophysiology and molecular mechanisms responsible for distraught in the immune system, which calls for more severe outcomes in patients with cardiovascular and hepatic defects[14]. In this article, we comprehensively evaluated the aspects concerning the invasion and spread of coronavirus as a tool to improve therapeutic strategies against liver damage in COVID-19 patients.

ORIGIN OF CORONA VIRUS: A BOTTOM-FEEDER OPPORTUNIST

Genus, Beta coronavirus isolated from COVID-19 patients, were identified using whole genome sequencing and matrix representation with parsimony phylogenetic analysis[15,16]. It was inferred that this virus was the closest relative of Rhinolophus affinis virus (bat CoV RaTG13) with more than 96% similarity[17,18]. Thus, the bat is projected to be its most suitable natural host. The phylogenetic interrelationship is depicted in Figure 1. Whole genome studies have revealed the presence of three SARS-CoV-2 strains A, B, and C among various human populations[19]. Originally strain A was found in the European population and is regarded as the ancestral strain closest to

the bat coronavirus[20,21]. At the same time, strain B observed some peculiar mutations which immunologically commit the virus to the East Asian zone[22]. Strain C, however, is more common among Americans, thus defining and tracking their outbreak areas[23]. Though the phylogenetic relationship is still abstruse for SARS-CoV-2, it is well established that the preferred reservoir host of this virus is the bat, which has spilled over to the man as a consequence of man-eating wild[18,24,25]. These are undoubtedly among the most prevalent RNA viruses (positive sense), which can afford a wide range of hosts[6,26]. Whole genome sequencing demonstrated that the SARS-CoV-2 virus is most closely related to the CoV RaTG13 virus[27,28].

After almost three years of corona panic, it is still disquietude as to what makes these viruses jump onto a human host and invade hepatocytes as a shared gateway. SARS-CoV and liver injury, along with upregulated transaminases in COVID-19 patients, indicate some strong link between the two[29,30]. SARS-CoV-2 has been deduced to possess a mosaic genome of different DNA sequences, comparable to 5 coronaviruses (probable donor strains) namely, Rhinolophus affinis RaTG13 coronavirus; R. pusillus RpYN06 viruses; R. pusillus BANAL-103; R.malayanusRmYN02 and R. malayanus BANAL-52 (analysed using genetic algorithm for recombination detection)[31-33]. The spike proteins of SARS-CoV-2 show more similarity with R. affinis RaTG13 and human angiotensin-converting enzyme 2 (hACE2) interaction is more related to R. malayanus BANAL-52. Another noteworthy feature is the absence of furin cleavage sites in all these viruses[34]. SARS-CoV-2 is one such unfortunate and deadly combination of disease severity and transmissibility among the viruses that it spilled over to humans, considering it a more prosperous and more lucrative host[35,36]. Coronaviruses are remarkable entities of 125 nanometres in size with one of the heftiest viral RNA genomes, accounting for 30000 nucleotides and equipped with an extraordinary power to correct drug-induced mutations due to outstanding proofreading ability[37]. The chances of getting a pandemic (zoonotic transmission/accidental spill-over) through exponential proliferation are also possible (chain of transmission to ensure its survival due to globalization and dispersal of the virus to far-off and susceptible populations).

Also, a new host like a human would lack previous immunity to the pathogen and so, in turn, secures a better board for the virus, providing a compatible incapacitating host defense mechanism host[38]. Above all, the increasing human-animal interactions (deforestation, hunting, domestication, wet market, wild animals as food preferences in many countries) further increase the chances of viruses to better adapt to human-modified niches[39]. This coronavirus is brilliant in its capacity to adapt to humans as hosts and, in the process, undergoes genetic evolution on account of new mutations. Conformingly, 5 SARS-CoV-2 strains are prevalent[40-44], as mentioned in Table 1.

Prognosis and pathophysiology: Thwarting effects of coronavirus on patients with existing liver diseases

Various factors govern the prognosis of this disease based upon pre-existing health conditions, comorbidity, age, course of treatment adopted, and patient's retort to treatment. The overall fatality rate strikes somewhere above 2%. Like most pathogens, the liver serves as one of the favorite proliferation spots for coronaviruses since it is a common gateway for viruses entering the blood[45,46]. It has been evaluated in many studies that around 1/3rd of COVID patients complained about liver dysfunction, and the cases were more frequent among male elderly patients[47]. A survey conducted on about 4000 seriously ill COVID patients confirmed an inflated mortality rate after three months, causing the death of more than 31% of the patients[48]. Even autopsies and biopsies performed (post-mortem) on the liver reflected that corona virus-2 inflicted cholangiocytes, hepatocytes, and endothelial cells, causing severe liver damage. Mechanisms of injury included hepatocyte ballooning majorly, eosinophilic action creating a cytokine storm, hypoxia, and ischemia leading to liver necrosis, as represented in Figure 2[49-51].

Hepatic fat accumulation indicating reasonable micro vesicular steatosis is a clear consequence of Corona virus-2[52]. Additionally, lobular inflammation and fibrosis cause severe liver cirrhosis. Cirrhosis further reduces blood flow through liver, thereby increasing blood pressure in the hepatic vein supplying from the intestine and spleen.

Once the Coronavirus enters the human body, major histocompatibility complexes ensure that highly pronounced pro-inflammatory (acquired and innate) responses are extended to tackle the uninvited viral guest[53-55]. SARS-CoV-2 uses ACE2 receptor to enter the target cells[56]. ACE2 are usually studded onto the bile duct epithelial cells, sinusoidal and capillary endothelial cells, and hepatocytes[57-59]. The viral antigen epitopes are recognized by antigen-presenting cells, majorly constituting macrophages and dendritic cells. These are finally presented to the CD4+ T helper cells, lymphocytes, and natural killer (NK-cells). These then further trigger the activation of B cells and CD8+ cytotoxic T cells[60-63].

The majority of the corona patients with dwindling survival rates have witnessed anomalous biochemistry, displaying fluctuating levels of some vital enzymes and biomolecules concerning hepatobiliary manifestations[64]. COVID-19 patients show dwindling hepatic functions, typically increased liver enzymes, ALT, and AST levels[5,65-67]. Several case studies attest to these findings in more than 50% of critically ill COVID patients[68]. Liver injury, chronic congestion, and nodular proliferation in corona patients are very likely, with more than 70% reporting steatosis[69,70]. The pathogenesis of hepatic damage in COVID patients is traced to multiple roots, including pre-existing hepatic disease, hypoxia, ACE-2 aided viral invasion and damage, ischemia, and drug-induced liver injury[71,72] as demonstrated in Figure 3.

MOLECULAR FEATURES: HEPATOTROPISM OR HEPATIC FRAILTY

The specificity of the virus to preferably invade hepatocytes is considered hepatotropism[65,73-75]. According to current research, SARS-CoV-2 does not specifically depict hepatotropism, while hepatocytes seem to be an easy target for people with a weak liver or compromised immune system[76,77]. Patients with some existing liver disorder display severe and prolonged symptoms of SARS-CoV-2 virus, as the immune system dysfunction displays a more pronounced effect[45]. Meta-analysis on more than 90000 COVID-19 patients pertaining to 40 case studies in United States and China alone attest to the hepatic deterioration[78]. Hence, the hepatic frailty makes liver cells more

susceptible and sensitive to COVID[79,80]. COVID-19 and comorbidities of hepatic diseases in a global perspective[80,81]. The invasion of SARS-CoV-2 virus in the human body, its genomic single-stranded RNAs, and the replicative double-stranded RNAs are sensed by cytosolic RNA sensors. These are then identified and bound to NOD-like receptors; endosomal toll-like receptors; melanoma differentiation-associated gene 5; and retinoic acid-inducible gene-I-like receptors[82]. These receptors then stimulate the next set of effectors molecules downstream interferon (IFN) regulatory factor 3/7 (IRF3/7); activator protein-1; and nuclear factor-κB (NF-κB). The next step encompasses the synthesis of pro-inflammatory cytokines, namely, IL-2, IL-10, IL-6, IL-8; and IFN-I, by activating its transcription. These IFN-I molecules are thus supposed to be the first line of defense to combat and clear the viral particles from the body; these thus induce signal transducer molecules the Janus kinase 1 (JAK1)/tyrosine kinase 2 and transcription 1/2 (STAT1/2), turning on the JAK1/TYK2-STAT1/2 pathway. This generates STAT1/2/IRF9 complex that additionally triggers transcription of IFN-stimulated genes. Thus, a cascade of events leads to the massive synthesis of antiviral chemicals: Procalcitonin; IL-6, CCL-5, IL-1, IFN-alpha, CXCL10, and CXCL-8, C-reactive protein[83]. Many studies have hinted at the unconventional triggering of certain supplementary systemic inflammatory responses leading to uncontrolled immune responses signaled by a storm of cytokines produced due to the activation of NF-κB and mitogen-activated protein kinase (MAPK) pathways[82]. This is commonly known as systemic inflammatory response syndrome, where a horde of immune cells together (B cells, T-cells, NK cells, dendritic cells, neutrophils, and macrophages) bring about a cumulative and exaggerated response[84]. Apoptosis and cell death remain the culminating stage regulated by the MAPK pathway. Pyroptosis is a specialized mechanism induced by coronaviruses to prevent viral spread leading to an inflammatory caspase-1-dependent cell death in patients in response to rapid viral replication within infected cells[85]. In this activation process, the virus secures its persistence through the PI3 kinase/Akt pathway[86].

PHARMACOLOGIC THERAPIES SPECIFIC TO CORONA-ASSOCIATED HEPATIC MORBIDITIES

Scientists worldwide could conceive fairly early the devastating effects of SARS-CoV-2, and social distancing was the only way out since impending outcomes were far from the view[87,88]. As an opportunist virus, it intimidated the whole world, shutting down everyday life and hampering the economy and health worldwide. Though we have successfully tamed this dangerous and feral pathogen, the efficacy of existing vaccines and drug therapies in preventing SARS-CoV-2 variants is still a matter of concern[87-89]. Vaccines were tracked on the plan to target spike proteins of the SARS-CoV-2, which the virus variants inventively cons[90-92]. Several novel vaccines, as well as drugs, have ardently helped in tackling these viruses. Since the virus shows high transmissibility and the future modulation in these viruses is erratic and unforeseen[90,92,93], prevention and management strategies should entail a multi-omic, closed-loop follow-up and holistic approach comprising scientists, government authorities, clinicians, pharmacists, and as the general public. Thus, prevention and management, including pharmacologic therapies against COVID-19, have been worked out under different approaches certified under Emergency Use Authorization[94-96]. Table 2 shows the currently used effective vaccines developed and successfully reduced morbidity and mortality across the world. Several therapeutic strategies are followed depending on the patient's condition as diagnosed by the clinician[97,98].

Immuno-modulators and anti-inflammatory agents

More appropriate to provide in the later stage of COVID infection. Clinical trials conducted on 113 COVID patients critically suffering from this disease, with both Baricitinib (inhibitor of Janus kinase) and Anakinra IL-1 antagonists on 52 COVID patients), have shown promising results in the case of COVID patients facing hyperinflammation (cytokine storm). These offer a dual inhibitory effect by preventing both entries of SARS-CoV-2 and preventing an exaggerated cytokine response[99-102].

Such trials have attested to the efficacy of critical-stage COVID-19 patients, especially those with hepatic complaints[103,104].

Anti-viral drugs

These are more suitable during the early phase of corona infection. Molnupiravir: A very effective drug that reduces both morbidity and mortality; paxlovid: Reduced 89% mortality (trial conducted on 1219 patients); remdesivir, hydroxychloroquine, lopinavir/ritonavir, ivermectin, and chloroquine are all Food and Drug Administration approved, but shows little or no effect over coronavirus; it is even not effective against corona variants[113,114]. Therefore, they are not recommended in case of patients with hepatic trouble during COVID-19. Also, some of these drugs (lopinavir/ritonavir, mitifovir, and tocilizumab) are not recommended and prescribed to patients with pre-existing liver diseases as these are known to cause cholestatic toxicity and hepatic injury[115-117].

Neutralizing antibodies against SARS-CoV-2

Antibodies naturally produced by the body of recovering patients or stimulated through vaccination can block the attachment and hence the entry of an enveloped viral pathogen inside the cell, conferring lifelong immunity[118-121]. Convalescent plasma transfusion therapy with a high anti-SARS-CoV-2 immunoglobulin G (IgG) titer effectively lowered the mortality of critical COVID patients[122-124]. The Bamlanivimab and Etesevimab antibody combination has been found to be super effective in COVID-19 patients with 87% lower death rate[125]. Another antibody cocktail, REGN-COV2, constitutes a group of two IgG1 antibodies (casirivimab and imdevimab) that target the receptor binding domain of SARS-CoV-2 and thereby reduce both morbidity and mortality of COVID patients by 70%[126].

CONCLUSION

Extraordinary efforts have reaped fruits. As per World Health Organization report on COVID-19 Vaccine Implementation Analysis & Insights, 63.4% of the World's population today stands vaccinated against COVID-19 in September 2022 (https://www.who.int/publications/m/item/covid-19-vaccine-implementation-analysis-insights-2-september-2022) and India alone proudly puts up 68% of its population in the list of a fully vaccinated cluster. This was made possible due to the untiring efforts of clinicians and researchers braced and heavily funded by the government and private agencies to curb this callous pandemic. It is anticipated that once 100% global vaccination is achieved, the virus will no longer be felonious. However, there are reasons to negate this notion. One explanation is that despite marshy governmental efforts, many people are vaccine-hesitant for inexplicable motives which may hamper virus block[127,128]. Even if this temper is somehow overcome, the dynamics remodelling human immunity to ongoing viral mutations and evolution is worth consideration. The co-evolution may equip the virus with new immune strategies to escape the human immune defence mechanism and maintain its virulence. According to the United States Centres for Disease Control and Prevention, viruses with new mutations are specifically a matter of concern and shall not be considered lightly[128]. Lifestyle, assess to wet markets, climate change, and increased animal-human interactions offer preferred gateways and richer niches to these evolving viruses[129]. The armchair experts in virology, immunology, and genetics have a lot to contribute to future mitigation strategies. It can no longer be one bug, one drug approach. Herd immunity is though expectantly looked upon but is short-term and modulates the virus with new attacking feats. What is good to know is that though the future is unseen, this COVID pandemic has taught us valuable lessons and equipped the health agencies, clinical experts, and the general public to face the subsequent pandemic terror. Masking and quarantines, lock downs, and work from home have become the new normal across the world.

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