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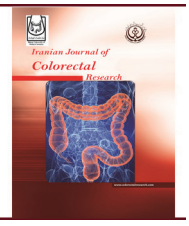

Where Do Liver Injuries Stand in the Post-COVID-19 Era? A Review of the Connection between Long COVID and Liver Diseases

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic threatened public health globally. Some patients who recover from the initial infection develop persistent symptoms and organ dysfunction for weeks or even months, called long COVID. Among multiple COVID-19-related complications, individuals may suffer from intrahepatic and extrahepatic complications principally mediated by ACE2 receptors. We reviewed PubMed, Google Scholar, and Web of Science manuscripts on underlying COVID-19-linked clinical relevance and potential pathogenesis of liver complications during short and long COVID with no time limitation. Liver impairment needs a large-scale and persistent follow-up as it may be multifactorial. During COVID-19, physicians must assess whether hepatopathy is associated with hepatic disorders, medications utilized for COVID-19 therapy, or viral antigenic outcomes progression to a complicated course. In the context of COVID-19, physicians report that potential pathophysiological approaches to hepatic failure in critical patients could lead to deep vein thrombosis, myocardial infarction, venous thromboembolism, and acute kidney injury. These complications might be either reversible or irreversible, with extended manifestations that mostly occur due to long COVID in the post-COVID era. Moreover, pre-existing cardiovascular and digestive tract problems correlate with adverse clinical outcomes and the highest fatality rate. Potential drug-disease interactions adversely influencing COVID-19 subjects and persistent comorbidities must also be considered. Besides the upshot of exiting hepatic-associated comorbidities, the effect of non-fatal and endothelial liver lesions on outcomes of COVID-19 patients remains elusive and must be investigated further. Measures to protect against hepatic toxicity should be considered when managing COVID-19 patients.

Keywords: COVID-19, Liver Failure, Post-Acute COVID-19 Syndrome

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic was the sixth public-health emergency in recent decades resulting in a broader global crisis (1). The World Health Organization (WHO) specifically developed the phrase COVID-19 on 11 February 2020 for symptomatic patients with severe pneumonia arising from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (2). Full-genome sequencing of SARS-CoV-2 revealed 82% homology with human SARS-CoV-1; both SARS-CoV-1 and SARS-CoV-2 access the host cell through the transmembrane angiotensin-converting enzyme 2 (ACE2) receptor. Based on the latest investigations, the prevalence of gastrointestinal (GI) symptoms in COVID-19 patient can be associated with a higher risk for disease severity, including long coagulation times and raised liver serum tests versus cases without GI symptoms (3). Notably, hepatopathy was also reported during SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV) infections (4).

Although COVID-19 complications have usually been reported to be reversible, they might sometimes represent irreversible patterns. Some patients who recover from the initial infection develop persistent manifestations lasting weeks or even months—conditions called "long COVID" or "post-COVID syndrome" (5). Regarding the duration of manifestations, long COVID is divided into two phases: post-acute COVID (symptoms lasting more than three weeks but less than two months) and chronic COVID (symptoms lasting more than two months) (6). Long COVID can be continuous or relapsing in nature (7), depending on the period between viral recovery and clinical recovery (8). The severity of the infection determines how much time is needed for clinical recovery (9). The exact mechanism(s) contributing to the persistence of manifestations are not fully understood; this persistence might be the consequence of immune response disruption, autoantibody formation, underlying diseases, unsafe hospitalization, persistent viremia, etc. (10). Long COVID can also be due to organ injuries; extents of organ defect along with the time needed for organ recovery are two substantial contributors in this field.

Hepatic function anomalies represented by dramatically elevated liver enzyme serum activities, prolongation of prothrombin time (PT), hyperbilirubinemia, and hypoalbuminemia may indicate a poorer prognosis in COVID-19 patients (11). Bangash and colleagues reported that impaired biochemical liver function tests (LFTs) were detected in 58% to 78% of patients with critical COVID-19, differentiating them from milder cases. Patients with severe COVID-19 appear to have more frequent signs of liver dysfunction than those with milder disease (12). Therefore, clinically major hepatic failure may be a considerable characteristic of the prognostic prediction of COVID-19 (13). Moreover, liver injury can occur in the post-COVID era, with or without pre-existing liver damage, and might be irreversible (14). A hepatopathy is potentially a multifactorial event (15), which most often is associated with viral hepatitis (due to chronic virus replication), ischemic hepatitis (due to lack of oxygen supply secondary to respiratory distress), invasive mechanical ventilation (due to high concentrations of positive end-expiratory pressure [PEEP]), drug hepatotoxicity (due to repurposing of antibiotics, antiviral, anti-malarial, and steroids drugs), antiviral immunity (via induced rapid generation of intrahepatic cytotoxic T cells and Kupffer cells), and/or altered gut barrier integrity and intestinal microbiota (16).

In the context of cellular and molecular mechanisms contributing to the pathogenesis of SARS-CoV-2-induced liver injury, multiple processes such as oxidative stress, hypoxia, cytokine storm, impaired immune response, mitochondrial dysfunction, autophagy, and the Unfolded Protein Response (UPR) are listed (17). Although the involvement of autophagy and UPR is not fully understood, they have recently attracted much attention in post-COVID hepatopathies. The inability to provoke hepatic autophagy during critical diseases, such as COVID-19, could deteriorate liver injury by promoting hepatic mitochondrial defect, thus affecting the UPR (18). In this regard, endoplasmic reticulum (ER) stress and its downstream response, i.e., UPR, and the autophagic flux, have been proposed to induce de novo lipogenesis in liver cells, which could sometimes be persistent (19). Thus, SARS-CoV-2 may promote hepatic steatosis through an ER stress-UPR/autophagy nexus (17). Additionally, autophagy has been reported to correlate with ACE2, as it is partially suppressed under the ACE2 action (20).

For the most severe acute cases of COVID-19, acute hepatic dysfunction seems consistent with higher rates of coagulation factor activation and fibrinolysis paralleled with thrombocytopenia (15). Although the full spectrum of clinical features and objective findings of patients with SARS-CoV-2 infection may vary according to time, the clinicopathological signs and mechanisms behind hepatic injury remain elusive (21). This review summarizes recent investigations to determine the clinical relevance and potential pathogenesis of liver complications concerning SARS-CoV-2 infection and post-COVID syndrome.

Hepatic Malfunction in COVID-19 Subjects

The phenotypic effect of invasive microorganisms on the liver can vary with enhanced liver function in host-exaggerated immunity against systemic infections by considering its predominant involvement in both hepatic portal and systemic circulations. The virus-associated cytopathic influence on hepatocytes and cholangiocytes causes cellular oxidative stress due to a diminished oxygen supply or increased

proinflammatory cytokines following SARS-CoV infection (22). RT-PCR analysis of SARS-CoV-2 RNA sequences isolated from the liver tissues of autopsied COVID-19 cases revealed the largest viral load of genome copies per gram of tissue, with 41% liver involvement (23). Pathologic signs on biopsy included hepatocellular necrosis, mitoses, cellular infiltration, and fatty degeneration (23). Furthermore, moderate microvesicular steatosis and mild portal and lobular activities were observed in liver tissue autopsies from a COVID-19 patient with sepsis or drug-induced liver injury (DILI) (24). Other key histopathological resources obtained from postmortem liver autopsies of two COVID-19 patients included mild zone sinusoidal dilatation, patchy hepatic necrosis, and a mild increase in sinusoidal lymphocytes (25).

As noted above, liver damage may be associated with direct viral infections (26). However, whether SARS-CoV-2 replication has direct adverse effects on liver function is still unknown. On the other hand, liver dysfunction can manifest following recovery from COVID-19, with or without a history of liver injury during the acute phase of COVID-19. This post-COVID hepatic malfunction may last for weeks or undesirably for months, threatening life quality (14). Fortunately, most patients exhibit mild liver impairments and return to normal conditions (27), but hepatoprotective therapy is required for those suffering from severe hepatic damage (28).

Both SARS-CoV-2 and SARS-COV-1 use the ACE2 receptor *via* the spike protein to enter the host's endothelial cells and enterocytes, minimally entering cholangiocytes. However, based on previous single-cell RNA-seq data, hepatocytes and Kupffer cells do not appear to accommodate the virus (29, 30) directly. Correspondingly, single-cell lineage tracing technology in combination with immunohistochemistry (IHC) techniques has demonstrated a relatively low expression pattern of ACE2 (0.31%) in biliary duct cholangiocytes of healthy hepatic tissues and slight ACE2 expression in diseased hepatocytes (29). The transgenic mouse models of severe hepatic impairment have also suggested an up-regulation of ACE2 expression in hepatocytes originating from bile duct epithelial cells, which readjusted to normal levels when liver function was restored, and hepatocyte proliferation was blocked (21, 30). Hepatic impairment, as the first atypical clinical manifestation of acute COVID-19, is extremely rare. However, liver toxicity in COVID-19 offers another possible pathophysiology (21). Besides virus-induced liver injury, immunological signals, systemic inflammatory response syndrome (SIRS), cytokine storms, hypoxic cell sensitivity (ischemia/reperfusion), and DILI could be the dynamic processes resulting in hepatic malfunction secondary to SARS-CoV-2 infection (31).

Dynamic Processes Associated with Liver Malfunction following SARS-CoV-2 Infection

Cholangiocytes and Cholangiopathy

SARS-CoV-2 can invade alveolar epithelial type II pneumocytes (AE2), mediated by spike (S) protein binding to the ACE2 receptor; the virus can also invade other tissues with high levels of ACE2 expression (32). In this respect, an extensive ACE2 co-expression has been reported in absorptive and crypt enterocytes from ileal, clonal, and esophageal mucosa of the gastrointestinal tract, as well as hepatocytes, bile duct epithelial cells (cholangiocytes), cardiocytes, renal proximal tubule cells, urothelial bladder cells, and pancreatic islets (33). Literature has addressed that SARS-CoV-2 might attach to ACE2-positive cholangiocytes but not hepatocytes to induce a remarkable cytopathogenic event (34).

Cholangiocytes can regulate multiple hepatic characteristics, particularly regenerative capacity and acquired immunological reactions. Therefore, the tremendous disturbance of cholangiocyte function in relation to cholestasis exacerbates COVID-19-associated conditions. In support of this, a recent article points to the significant elevation of GGT in 54% of case series of SARS-CoV-2 infection (11, 35, 36). Deregulated expression of genes involved in cellular adherent plaque-forming junction and bile acid transportation has been identified in the human ductal liver model with an enhanced vulnerability of cholangiocytes following functional impairment of barrier and canalicular bile network in COVID-19-associated hepatic failure (35). Among a specific subpopulation of vertebral transmembrane glycoproteins, there are two species of Neuropilins (NRPs), NRP1 and NRP2, with similar domain structures composed of a large N-terminus hydrophobic residue and a small C-terminus one toward the cytoplasm (Figure 1) (29).

Post–COVID cholangiopathy is a particular type of liver impairment described as 'secondary sclerosing cholangitis (SSC) - critically ill patients (CIP) (37). SSC-CIP is a newly identified form of cholestasis, especially in those without a history of hepatobiliary defect, following hospitalization and receiving therapeutics in the intensive care unit (ICU) under various conditions such as trauma and infection (38). In the case of COVID-19, prolonged hospitalization due to acute respiratory distress was reported to be the reason for developing cholestasis with correlated jaundice that persisted even after pulmonary recovery (37). Mechanistically, many predisposing factors have been reported to be responsible for SARS-CoV-2-induced SSC-CIP, in which low blood pressure and consumption of vasopressors are hallmarks. Cytokine storm due to the elevation of proinflammatory cytokines can also result in the progression of bile duct toxicity and, thus, cholangiocyte necrosis (39).

Interestingly, the histopathological signs of post-COVID cholangiopathy appear to differ from the histopathology of SSC-CIP of other origins (40). For

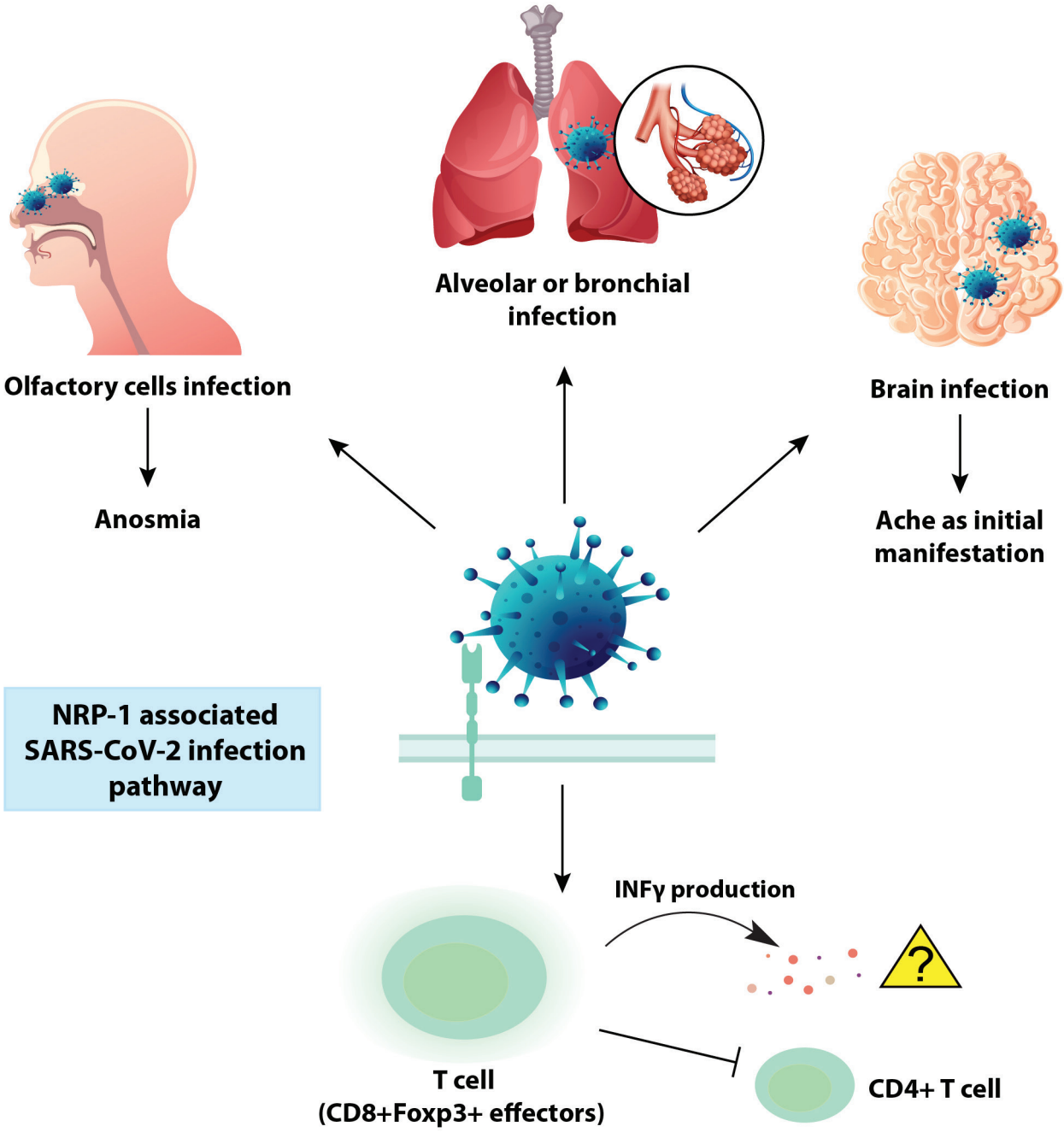

Figure 1: The plausible pathway of Neuropilin (NRP): The conjugation of the b1b2 ectodomain of NRP-1 to the viral Spike (S) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an ACE2-unrelated pathway for SARS-CoV-2 entrance and infection of NRP-1-positive brain parenchyma and olfactory nerves, resulting in anosmia and headache along with respiratory system infection (alveolar, bronchial). Comparatively, NRP1 upregulates primary effector and memory CD8+ T cells vs. naïve cells and controls the output of interferon (IFN)-α via an undetermined mechanism following viral infection. CD4+ T cell proliferation is suppressed after NRP1 upregulation in CD8+Foxp3+ effectors in infected intestinal mucosa. Non-specific hepatic antigen-presenting cells (APCs) stimulate the synthesis of distinct NRP1+CD8+ memory T cells. During physiologic conditions, peripheral foreign antigens resist primary cytotoxic T-cell (CTL) lysis after the presentation to lymphoid tissues mediated by sinusoidal endothelial cells. Subsequently, those primary CTLs stimulated by non-specific APCs remove peripheral colonized pathogens that had evaded the first line of defense. Regardless, the performance of NRP1 in primary CTLs remains unclear.

instance, the biopsy specimens indicated remarkable degenerative cholangiocyte damage accompanied by cholangiocyte cytoplasmic vacuolization that was not previously reported for SSC-CIP (41). Indeed, these histologic alterations propose direct liver impairments due to COVID-19 in subjects with underlying SSC-CIP (37).

Autophagy and Liver Injury

Studies on liver autopsies from COVID-19 subjects have demonstrated that hepatic steatosis, as the intrahepatic accumulation of fat content, might be the most critical risk factor for liver injury in COVID-19 and post-COVID hepatocellular infection (42). Understanding the difference between SARS-

CoV-2 infection-induced steatosis and pre-existing non-alcoholic fatty liver disease (NAFLD) is crucial to decreasing the risk of poor results in patients with COVID-19 (36). Among different possible mechanisms attributed to COVID-19-related hepatic steatosis, deregulation of lipid metabolism, as well as mitochondrial dysfunction due to SARS-CoV-2's cytopathic impact and cytokine storm-triggered immunopathy are considered the key contributors to infection-associated lipid accumulation (17). Interestingly, the mammalian target of rapamycin (mTOR), as a substantial modulator of the autophagic flux, is involved in the host lipid metabolism as a lipogenesis inducer (19, 43). Previous findings have shown that SARS-CoV could induce autophagy through highly conserved processes in correlation with non-structural protein 6 (nsp6) (44). Rapamycin-induced induction and suppression of the mTOR signaling have been demonstrated to block viral replication in the MERS-CoV-infected HuH7 cell line (45). Thus, it can be concluded that SARS-CoV-2, like other coronaviruses (i.e., SARS-CoV and MERS-CoV), has a common mTOR-based mechanism of infection (46).

SARS-CoV-2 infection can induce hepatic mTOR signaling pathway through hepatocellular disease and/or IL-6-dependent cytokine storm-associated outcomes, which could finally result in steatosis in those with COVID-19 liver dysfunction (17). Although the overactivation of lipogenic processes is vital for SARS-CoV-2, it significantly hurts the host. Indeed, de novo lipogenesis, by providing significant amounts of lipids, makes the virus able to generate essential vesicular systems for replication and exocytosis (17). Furthermore, the induction of mTOR-mediated protein biosynthesis and the suppression of autophagolysosome formation can trigger viral replication while inhibiting viral degradation and immune response initiation (17, 47). The higher risk of more severe COVID-19-associated consequences in patients with obesity and diabetes mellitus may be because insulin and glucose signaling pathways upregulate hepatic mTOR, leading to subsequent liver complications such as hepatic steatosis (17, 48, 49).

Autophagy has also been reported to be limited by a virus-triggered AKT1-dependent activation of the SKP2, an E3-ligase S-phase kinase-associated protein, in MERS-CoV-infected cells. Likewise, recent evaluation has demonstrated the overexpression of autophagy receptor SQSTM1/p62 in SARS-CoV-2-infected cells, which mildly suppresses the autophagic flux (50). This viral infection also stimulates AKT1/SKP2-dependent autophagy to promote the degradation of Beclin-1 (51). All these findings collectively propose that SARS-CoV-2 infection could limit the process of autophagy.

ACE2, the key SARS-CoV-2 receptor, which is highly expressed in alveolar epithelial cells, is also expressed in liver endothelial cells, thus converting the liver into a target organ for SARS-CoV-2 (52, 53). Surprisingly, ACE2 is in correlation with autophagy and its role in mediating the entry of novel coronaviruses into host cells; autophagic flux is partially suppressed under the ACE2 action (20, 54). The overexpression of ACE2 has been detected in autophagy-deficient cells (55). Taken together, COVID-19 can also induce liver injury, probably by disturbing the autophagic flux in an ACE2-dependent manner (50). This type of liver injury can manifest either during or after the infection.

Unfolded Protein Response Linked to Hepatic Function Defects

Epithelial cells lining the upper and lower respiratory tract are the major targets for SARS-CoV-2 invasion, and viremia only occurs in patients with severe infection (56). The formation of viral proteins in infected cells and subsequent ER stress may disrupt proteostasis in host cells, suppressing functional protein synthesis and thus inducing cell apoptosis (57). According to the Sequential Organ Failure Assessment (SOFA) of patients who died from COVID-19, liver dysfunction and multiple organ failure were commonly present (25, 58, 59). Direct viral infection results in diffuse vascular endothelial inflammation in the liver and other organs such as lungs, kidneys, heart, etc. (60). Although the elimination of viral particles or infected cells would cause a "cure" for the infection, SARS-CoV-2 might carry on the proliferation where cellular dysfunction can be tolerated by the host's UPR (57). Furthermore, proteomics evaluations of multiple organs from patients with COVID-19 have demonstrated that ER stress is increased in liver cells and the lungs, resulting in the engagement of UPR (61).

Stress-triggered autophagy has a crucial role in cell survival and decreasing the undesirable effects of ER stress (62, 63). *In vitro* analysis has revealed that activating transcription factor 6 (ATF6), a key axis of the UPR, positively regulates Beclin1 and autophagy-related gene 9a (Atg9a) (64). Besides, the eukaryotic translation initiation factor 2 alpha kinase 3 (EIF2AK3), as another UPR axis, can promote the expression of multiple autophagy modulators such as Atg5 and Beclin1 (65). X-box-binding protein 1 (XBP1) also acts similarly by activating Beclin1 (66). Considering the transcriptional interaction between ER stress and autophagy in association with XBP1-mediated activation of transcription factor EB (TFEB), as a key modulator of lysosomal dynamics, Zhang et al. established that loss of this interaction could disrupt hepatic autophagy in metabolic disorders such as obesity. They found that obesity-dependent down-modulation of XBP1-induced activation of the TFEB was central to disrupted autophagy in the liver (67). However, the current mechanism has not yet been attributed to SARS-CoV-2-related liver injury, and further explorations are still needed to clarify the exact crosstalk between

UPR and SARS-CoV-2-induced hepatic damage.

In addition, ER stress is also considered to provoke de novo lipogenesis in liver cells (68). Interestingly, the up-modulation of glucose-regulated protein 78 (GRP78) and GRP94, prominent ER stress markers, has been detected upon SARS-CoV-2 infection in multiple cell lines (17). On the other hand, the coronavirus S protein can promote ER stress (69, 70). Once ER stress is triggered, the relevant PERK-eIF2-α pathway, as a key UPR arm, is hyper-activated by SARS-CoV-2 (71). Thus, SARS-CoV-2-induced ER stress could stimulate de novo lipogenesis, leading to hepatic steatosis in COVID-19 subjects or those recovered from the infection, mostly through the aforementioned UPR-associated pathway (Figure 2) (17).

Systemic Oxidative Stress and Liver Dysfunction

Extraordinary activation of inflammatory mediators, especially aberrant blood amounts of C-reactive protein (CRP), lymphocytes, neutrophils, and cytokines, mostly interleukin-6 (IL-6), leads to subsequent innate immune response deregulation that may advance to respiratory failure and extrapulmonary manifestations such as liver injury during and also after COVID-19 (72). In normal physiological states, the liver is the major immunological site, which collects and screens many excreted substances, constituting immunological tolerance over the intestine-hepatic axis (73). However, disrupted immunological tolerance through hyperactivated immune responses and cytokine storms during COVID-19 could cause a catastrophic insult to internal organs, especially the intestine and liver (73, 74). Meanwhile, liver injury could be elicited by hypoxia-reperfusion, effectual hyperactivation of Kupffer cells under oxidative stress, intestinal endotoxemia, and the existence of adrenergic autonomic nerve activation in COVID-19 patients. Sepsis is not common in acute and urgent COVID-19 patients, notably in the presence of dysbiosis of the intestinal microbiota with liver cirrhosis; however, it might be one of the predisposing factors for acute hepatic insult and adversely affects the prognosis of these patients (75).

Septic shock is initiated by impaired function of the systemic response to an infection, which in turn strongly mediates psychological stress and sequential organ dysfunction. The physiopathology of hepatotoxicity–associated septic shock involves collateral hypoxic liver injury from viral-induced ischemia and shock, cholestasis after altered bile acid homeostasis, hepatocellular toxicity congestion from drug poisoning, or an inordinate inflammatory response (76). Furthermore, acute hypoxia and profound volume-depleted abnormalities are the primary drivers of hypoxic-ischemic (HI) insult hepatitis in COVID-19 patients with severe respiratory malfunction and/or distress. Hypoxic-ischemic hepatitis is frequently linked to metabolic acidosis, resulting in the highest serum concentrations of hepatocellular aminotransferases (75, 77, 78).

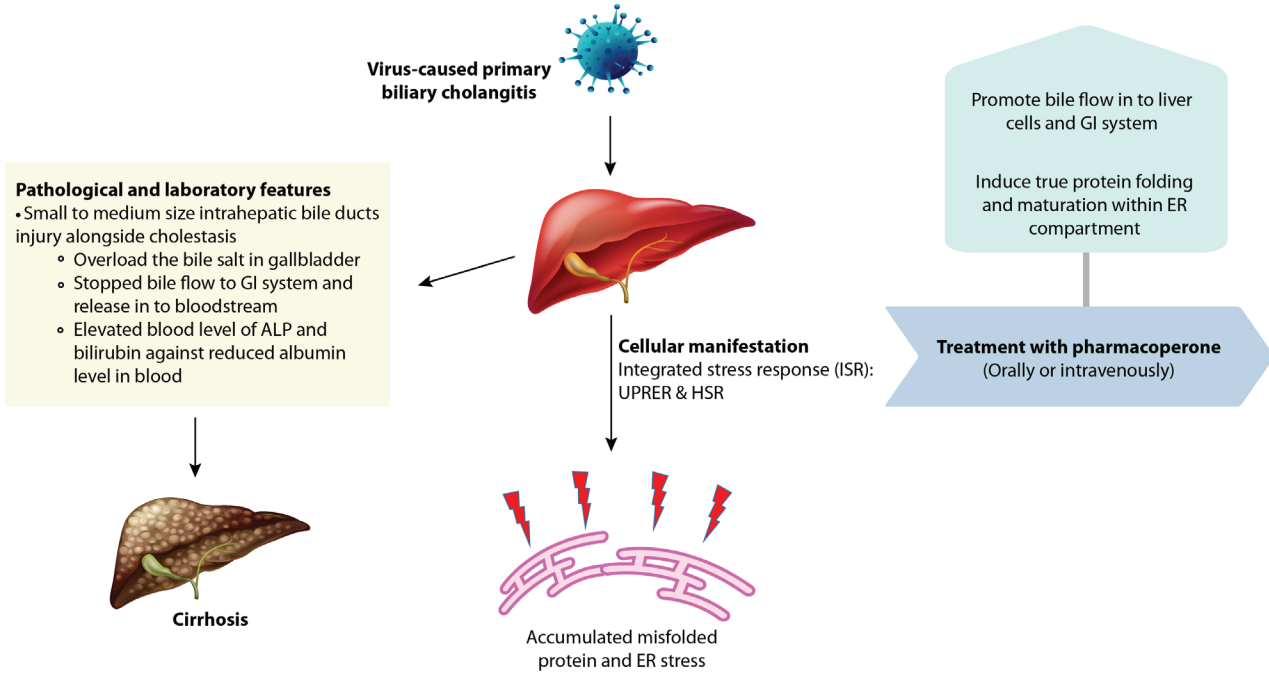

Figure 2: The association between the ER packaging system and primary biliary cholangitis; The mass of mispacked proteins, accompanied by stiff integrated stress response (ISR), the unfolded protein response (UPRER) and intracellular heat-shock response (HSR) responses may provide a therapeutic advance in COVID-19 medication. In primary biliary cholangitis, pharmacoperones like ursodeoxycholic acid (UDCA), TUDCA/UDCA compositions, 4-phenyl butyric acid (PBA), and tauroursodeoxycholic acid (TUDCA) (orally or intravenously) promote true protein folding, maturation, enveloping, and modification in the endoplasmic reticulum (ER), mitigate ER stress, and prevent cellular malfunction, inflammatory stress, and the induction of proapoptotic effects. TUDCA has been researched preclinically and clinically. As a hydrophilic synthetic taurine-conjugated derivative, UDCA, like other pharmacoperones, fosters the flow of canalicular bile salts into liver cells and other parts of the gastrointestinal system.

Drug-induced Liver Injury (DILI)

The liver is the site of the metabolic breakdown of various drugs, such as newly explored nucleoside analogs and protease inhibitors with the potential to manage COVID-19 (79). Moderate microvascular steatohepatitis, benign lobular inflammation, and hepatocellular insult have been seen in some patients with a history of receiving antipyretics (due to paracetamol-related liver toxicity), signifying the risk of drug-induced hepatotoxicity (21). Additionally, several COVID-19 patients with a history of receiving antiviral medications like oseltamivir, idol, lopinavir/ritonavir, as well as antitumor drugs, antituberculosis drugs, and antimalarial drugs presented with liver toxicity (80). Accordingly, anomalous elevated amounts of Aspartate transferase (AST) and alanine transaminase (ALT) accompanied by high total bilirubin levels appeared as side effects in a few symptomatic COVID-19 subjects, which subsequently raised the stakes for hepatotoxic lopinavir and ritonavir drugs (55.4%) (22).

Another retrospective case series on COVID-19 declared that the utilization of these pairwise drugs was much higher in cases with atypical LFTs versus individual patients without altered LFTs (56.1% vs. 25%, P=0.009) (22). An increasing trend for abnormal baseline LFTs accounted for 47.3% of all discharged cases, and 23.7% developed anomalies during the hospital stay (22). However, long-term hospitalization associated with LFT abnormality in these cases was much less common than in acute or critical cases. The study revealed that CYP3A4 substrates are essential in ritonavir-mediated hepatotoxicity through CYP3A metabolic pathways and oxygen-free radical generation. CYP3A4 substrates covalently bind to membrane lipids and thus induce lipid peroxidation, loss of membrane integrity, cellular internal and external Ca^{2+} homeostasis disruption, and influence ER stress and mitochondrial dysfunction, which ultimately lead to hepatocellular injury and eventually cell death (79). In conformity with the literature, the autophagy signaling and UPR associated with ER stress could be a good objective for confronting COVID-19 affliction.

Apart from reported rare hepatotoxicity caused by exposure to chloroquine or hydroxychloroquine, these conventional drugs with practicable pharmacodynamics for current therapeutic indications stopped cytokine storms, $CD8^{+}$ cells induction, or caveolae-dependent endocytosis by interrupting the cellular entry of the virus (81). Tocilizumab is an anti-human IL-6 receptor inhibitor, which has been utilized as a substantial therapeutic in COVID-19 patients with excessive cytokine release. However, a mild increase in LFTs frequently determined over the period of 2–6 weeks post-exposure was reported in other indications (82). The experimental antiviral nucleotide, remdesivir, showed no documented evidence of liver toxicity (83). A series of well-designed studies have noted that fluoroquinolone (levofloxacin) and quinolone antibiotics, alone or in combination with antiviral medications such as ribavirin, steroids, and other novel agents utilized empirically to improve COVID-19 outcomes, could lead to hepatic impairment (84).

One of the major alternative causes of an abnormal hepatic panel during the COVID-19 era is the population with a history of pre-existing liver disorders, including NAFLD, chronic hepatitis B, and liver cirrhosis. Uncontrolled immune responses triggered by SARS-CoV-2 can act as a "second hit" to simple fatty liver, leading to liver injury and steatohepatitis (21). Oxidative stress and septic shock worsen the already poor prognosis in cirrhotic liver conditions and activate the transition from acute to chronic hepatic impairment (85). Gut microbiota alterations also affect the incidence and progression of pre-existing liver diseases like liver cirrhosis, alcoholic fatty liver disease (AFLD), and NAFLD (86).

Long-term Hepatic Side Effects Post-COVID Recovery

Increased LFTs have been reported with abnormal levels of AST and ALT in post-COVID circumstances (87). Most COVID-19 patients develop only mild hepatic malfunction and return to normal conditions; however, hepatoprotective therapy is required in those with severe injury (28).

Among 192 COVID-19 hospitalized patients, Zhan et al. reported liver damage in 39% upon admission, while 69% experienced liver impairment through hospitalization. In patients with severe COVID-19, liver damage was detected in 86% (88). In another study by An and co-workers, 253 discharged patients with hepatic recovery underwent a two-month follow-up evaluation; 20.2% of those had chronic liver disorders, including hepatitis B, fatty liver, and cholecystopathy, before being infected. These patients experienced higher degrees of liver injury than the remaining 79.8% during hospitalization (89). The hepatic serological state of infected patients at two weeks post-discharge was also compared with healthy subjects in the same study; total protein content, albumin levels, albumin-to-globulin ratio (A/G), and the De Ritis ratio (AST/ALT) were markedly lower in patients against healthy individuals. On the other hand, GGT and ALP levels were remarkably higher in infected cases. Once the serologic tests were evaluated 40 days post-discharge, ALT and GGT levels decreased to less than 10% (89).

Multifaceted metabotyping demonstrated that various parameters, such as taurine, increased while the glutamine/glutamate ratio decreased in post-COVID cases versus healthy subjects (90). Thus, suitable interventions and liver function repair are necessary for COVID-19 patients. Chronic liver conditions carry higher risks and thereby require special interventions.

Patients with Pre-existing Chronic Liver Disease and Hepatocellular Carcinoma

To treat cases of liver damage, it is recommended to use drugs that can stop inflammatory responses and preserve liver function. Evidence shows that patients with chronic liver diseases (CLDs), including hepatocellular carcinoma, decompensated cirrhosis, non-alcoholic fatty liver disease, liver transplant, or autoimmune liver diseases, may have a higher risk for severe infection (91).

Recorded data from CLD patients indicate that patients with compensated cirrhosis during SARS-CoV-2 infection are at increased risk for decompensation and death, even without respiratory disorders (92). Interestingly, the contribution of thromboembolic disorders to developing serious hepatic complications in pre-existing CLD patients has been suggested (93). A large cohort study, including 2,780 COVID-19 patients, showed a higher death rate in patients with pre-existing liver disease than patients who did not have liver disease. The authors reported that comorbidities were higher in patients with liver disease, and this proportion was 48% for diabetes and 68% for hypertension (94). Another study showed that 43–63% of patients with decompensated cirrhosis did not survive, while the mortality rate in patients with liver disease but without cirrhosis was 12% (95).

Generally, more research is needed to understand the reason for liver damage in severe COVID-19 patients because ACE2 receptors are not present in hepatocytes. Therefore, the effects of pre-existing liver disease on COVID-19 therapy should be determined.

The most vulnerable group in COVID-19 is known to be cancer patients. Indeed, various methods of anti-cancer treatments cause severe systemic immunosuppression and increase disease susceptibility. Although not much is known about Hepatocellular carcinoma (HCC) and COVID-19, cancer patients appear to be at an increased risk for COVID-19 and poorer outcomes compared to those who do not have cancer. COVID-19 may exacerbate CLD in patients with HCC and make cancer management more difficult. Physicians believe that modification of management and deviation from the standard of care during HCC management is essential during and post-COVID-19. Treatment deferment is the first clinical practice modification to decrease the prevalence of COVID-19 among cancer patients (96). The lack of anesthesia facilities and ICU beds during COVID-19 reduces the capacity of centers for surgery (97). In addition, with a lack of donors and reduced anesthesia capacity, liver transplant services for HCC may be reduced or temporarily discontinued. In centers where radiology and oncology services are less affected by the pandemic, transarterial treatment, systemic therapy, and radiation therapy may be considered for HCC (98). As delaying anti-cancer treatment is not a sensible option to decrease the infection risk in patients, more detailed studies of risk factors for cancer patients in the pandemic or post-pandemic era need to be performed, and physicians should report appropriate COVID-19 reports in patients who had malignancies.

COVID-19 Drug Treatments and Their Role in Liver Injury

There are currently some approved drug treatments for COVID-19. According to Emergency Use Authorization (EUA), the Food and Drug Administration (FDA) has issued the use of three main therapies, including remdesivir, chloroquine or hydroxychloroquine, and convalescent plasma for patients hospitalized with severe COVID-19 (99) .Considering the quickly developing knowledge about SARS-CoV-2, several potential drug targets have been proposed and are under investigation for SARS-CoV-2 infection. These investigational drugs are reviewed below.

Nucleoside Analogs

Remdesivir

Remdesivir, or GS-5734, is an adenosine analog inhibiting the RNA-dependent RNA polymerase. Investigations using in vitro culture cells, mice, and nonhuman primate (NHP) models have indicated that remdesivir is a promising antiviral drug against a broad spectrum of RNA viruses like Ebola and SARS/MERS-CoV (100). Grein J. et al. performed a case-series study on 53 patients with severe COVID-19 treated by remdesivir. They found gastrointestinal and hepatotoxicity as the most common adverse effects in these patients (100). Zampino and colleagues obtained similar results. According to their observation, remdesivir may have a direct role in hepatocellular toxicity and elevate the levels of ALT and AST. Therefore, they suggested that remdesivir application needs caution in patients with prior liver disease, and liver function should be closely monitored during the treatment by remdesivir (101). Different clinical trial studies are ongoing to investigate the antiviral efficacy and safety of remdesivir in mild, moderate, and severe COVID-19 patients (NCT04280705, NCT04257656, NCT04292899, NCT04252664, and NCT04292730) (102, 103).

Ribavirin

Ribavirin is known as a wide-range antiviral agent (104). This guanosine analog inhibits viral RNA-dependent RNA polymerase. Considering the in vitro activities of ribavirin against other coronaviruses, including SARS-CoV and MERS-CoV, it was suggested as a potential therapeutic candidate for COVID-19 treatment (105). The administration of ribavirin as monotherapy is limited since high concentrations are required to inhibit viral replication. Ribavirin causes hemolysis and liver

toxicity in a dose-dependent manner (100). The combination of ribavirin with lopinavir/ritonavir or interferon has been recommended for COVID-19 treatment (104). It should also be noted that the dual therapy of ribavirin and interferon is also used to treat hepatitis C virus (HCV) infection.

Previous systematic review and meta-analysis studies revealed inconclusive results regarding ribavirin's efficacy in treating SARS and MERS (106). Safety concerns exist concerning ribavirin's severe adverse effects, including hematologic and hepatic toxicity. Considering the inconsequent results of ribavirin efficacy for SARS and MERS and its toxicity, ribavirin offers limited value as monotherapy for COVID-19 patients (107).

Chloroquine and Hydroxychloroquine

Chloroquine and hydroxychloroquine appear to act as broad-spectrum antiviral drugs. They block virus/cell fusion by enhancing endosomal pH and inhibiting the glycosylation of cellular receptors, endosomal acidification, and proteolytic processing. Wang et al. evaluated the SARS-CoV-2 infection in Vero E6 cells, indicating that chloroquine acts on entry and post-entry stages (107).

Chloroquine and hydroxychloroquine attenuate cytokine production, inhibiting autophagy and lysosomal activation in host cells. Therefore, in addition to antiviral activity, these agents offer immune-modulating activity, which may synergistically increase their in vivo antiviral function (108). Considering the safety, low price, and antiviral efficacy of chloroquine and hydroxychloroquine, they have been suggested for treating COVID-19 (109). Although some studies have shown the efficacy of these agents in improving viral clearance and radiologic findings while stunting disease progression, further investigations with larger sample sizes are required to confirm these results.

Administration of chloroquine and hydroxychloroquine did not cause any adverse effects in most patients with COVID-19. The application of these agents is generally considered safe during pregnancy (109). However, rare but serious adverse effects have been reported after treatment by these agents (<10%), including hypoglycemia, retinopathy, neuropsychiatric effects, and cardiac conduction defects (109). Nausea, vomiting, diarrhea, and abdominal pain are rare gastrointestinal (GI) adverse effects induced by chloroquine and hydroxychloroquine. Both drugs are rated with a score of D by the National Institute of Health LiverTox resource, which considers them a rare possible cause of apparent liver injury (109).

Chloroquine is rarely associated with an increase in aminotransferase levels or hepatotoxicity. It may cause hypersensitivity attacks with fever, elevated serum aminotransferase, and jaundice in patients with intermittent acute porphyria or porphyria cutanea tarda. These adverse effects are less common in patients treated with hydroxychloroquine. Hydroxychloroquine is concentrated in the liver, so it should be cautiously administered in patients with liver diseases like hepatitis and those taking other hepatotoxic drugs (109).

Lopinavir/ritonavir

The FDA-approved combination of lopinavir/ritonavir is newly developed to prevent and treat human immunodeficiency virus (HIV). Considering the proteinase inhibitory function of lopinavir/ritonavir, which inhibits coronavirus 3-chymotrypsin-like protease, this combination has been suggested as a potent drug candidate against MERS and SARS (110). Evidence demonstrated decreased viral load and improved clinical symptoms in patients with COVID-19 treated with lopinavir/ritonavir (107). This combination should be administered during the early peak of viral replication (first 7-10 days) (92). Although some clinical studies on SARS indicated reduced mortality and intubation rates after lopinavir/ritonavir treatment, definitive conclusions were not reached due to their retrospective and observational nature. Gastrointestinal upset like nausea and diarrhea (up to about 28%) and hepatotoxicity (2–10%) are the main adverse effects of lopinavir/ritonavir (109). Considering that about 20–30% of patients with COVID-19 presented elevated transaminases, these adverse effects may be intensified with combination therapy or viral infection (111).

A previous study reported the critical role of the 3A4 subfamily of the cytochrome P450 superfamily (CYP3A4) in hepatotoxicity mediated by ritonavir (107). CYP3A metabolic pathways can create electrophilic compounds, which covalently bind to macromolecular materials within the liver cells and disrupt lipid metabolism, membrane integrity, and intra- and extra-cellular Ca^{2+} homeostasis, ultimately affecting the function of vital organelles like endoplasmic reticulum and mitochondria and even causing liver damage and death (112). Therefore, hepatotoxicity induced by lopinavir/ritonavir may limit the possibility of using this combination therapy.

Sirolimus (Rapamycin)

Sirolimus (SRL), also called rapamycin, and other mTOR inhibitors (e.g., everolimus and temsirolimus) have provided a possible approach to treat COVID-19 and ameliorate the clinical manifestations, as well as pathological complications (113). SRL blocks the proliferation of effector T-cells while triggering the accumulation of regulatory T-cells (Treg) (114). In an ideal manner, SRL may decrease immune-mediated injuries during COVID-19 without altering the defensive capacity of the immune system against other possible infections. More interestingly, SRL could also repress viral replication in SARS-CoV-2 and other coronaviruses (i.e., SARS-CoV and

MERS-CoV) by affecting an mTOR-based infection mechanism (46). It is also proposed that SRL attenuates lipogenic processes in relation to mTOR signaling, resulting in the amelioration of COVID-19-induced hepatic steatosis (19). Consequently, the early administration of mTOR inhibitors may exert protective effects against COVID-19 and prevent clinical deterioration. Although clinical evidence about SRL's therapeutic effects against COVID-19 is missing and many clinical trials are still ongoing, immunomodulation could represent a promising approach to overcome COVID-19-related liver injury.

Conclusion

In summary, hepatic injury is a typical clinical manifestation secondary to COVID-19, especially in those with multisystem organ dysfunction. According to histopathological investigations, hepatic injury in COVID-19 patients is characterized by microvesicular steatosis along with lobular and portal inflammation. SARS-CoV-2 has the potential to attack hepatocytes directly and cause hepatic malfunction through a cytopathic mechanism, according to autopsied liver biopsies. Molecular investigations also demonstrated the remarkable contribution of autophagic flux and ER stress-induced UPR to the progression of hepatic steatosis during and after COVID-19. Beyond steatosis, these two mechanisms could also promote liver damage, leading to hepatocyte death. Furthermore, the cytokine storm syndrome has been identified in severe COVID-19 patients. Individual consideration must be concentrated on the liver function status, notably on abnormal levels of ALT, AST, albumin, total bilirubin, direct bilirubin, and INR, and their effects among critically ill COVID-19 subjects.

In post-COVID cases with accompanying liver injury, specific caution should be paid to carefully identify primary hepatic impairment-related causes combined with the pathophysiological findings by hematoxylin and eosin morphology and more comprehensive analyses like transmission electron microscopy or antibody-based genomics/proteomics assays. It will be significant and noteworthy to detect the viral RNA originating from specific liver cells and to assess whether hepatocytes express the ACE2 receptor in a high volume of COVID-19 patients. Interestingly, ACE2 receptors are reported to correlate negatively with autophagy.

Measures to protect against hepatic toxicity should be considered when managing COVID-19 patients during and after acute illness. Besides the upshot of exiting hepatic-associated comorbidities, the effect of non-fatal and endothelial liver lesions on outcomes of COVID-19 patients remains elusive and must be investigated further. Regional data from each state is necessary to understand this multifarious disease comprehensively.

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Authors' Contribution

PM, SVH, and SI designed the concept. MS and SI prepared the figures. PM supervised the project. MZ and PM received the grants. All authors wrote the manuscript and read and approved the final version of the manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest

Dr. Seyed Vahid Hosseini, as the Deputy Editor, was not involved in any stage of handling or reviewing this manuscript.

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