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COVID-19 related liver injury: Focus on genetic and drug-induced perspectives

Parchwani D *et al*. Spectrum of COVID-19 liver injury

Abstract

BACKGROUND

Empirical use of potentially hepatotoxic drugs in the management of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is considered as one of the major etiopathogenetic factor for the liver injury. Recent evidence has shown that an underlying genetic factor may also concur. Hence, it is important to understand the host genetics and iatrogenic based mechanisms for liver dysfunction, so as to take timely remedial measures.

AIM

To investigate drug induced and genetic perspective for the development of coronavirus disease 2019 (COVID-19) related liver injury.

METHODS

Reference Citation Analysis, PubMed, Google Scholar and China National Knowledge Infrastructure were searched by employing the relevant MeSH keywords and pertaining data viz authors' duration, site and type of study, sample size with any subgroups, drug induced liver injury outcome, genetic aspect were extracted from most current pertinent publications.

RESULTS

In all studies, the hepatic specific aminotransferase and other biochemical indices were more than their prescribed upper normal limit in COVID-19 patients, and was found to be significantly related with the gravity of disease, hospital stay, number of COVID-19 treatment drugs, and worse clinical consequences. In addition, membrane bound O-acyltransferase domain containing 7 (MBOAT7) rs641738, rs11385942 G>GA at chromosome 3 gene cluster and rs657152 C>A at ABO blood locus was significantly associated with severity of livery injury in admitted SARS-CoV-2 patients.

CONCLUSION

Hepatic dysfunction in SARS-CoV-2 infection could be the result of individual drug or due to drug-drug interactions and may be a subset of patient population had a genetic propensity. Thus, serial estimation of hepatic indices in SARS-CoV-2 infection hospitalized patients should be done to take timely corrective actions for iatrogenic causes to avoid clinical deterioration. Additional molecular and translational research is warranted in this regard.

Key Words: SARS-CoV-2; Liver injury; Genetic prospective; Drug induced liver injury; Prognosis; COVID-19

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Core Tip: Scientific evidence highlights the multisystemic nature of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and hepatic dysfunction is the front and centre among the extrapulmonary manifestations. In addition to the direct cytopathic effect of the virus, iatrogenic causes and genetic susceptibility are also postulated in the pathogenesis of hepatic damage in SARS-CoV-2 infection. Degree of liver toxicity in terms of altered biochemical indices were consistent with severity of coronavirus disease 2019 (COVID-19) illness and hospital stay. Hence, serial monitoring of hepatic indices in COVID-19 hospitalized patients may provide useful prognostic value so as to take timely corrective actions to avoid clinical deterioration.

INTRODUCTION

Since the index case of coronavirus disease 2019 (COVID-19) infection was confirmed in the month of December 2019 in China[1], the upsurge of COVID-19 has led to devastating effects on global health[2]. Considering the incessant evolution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its impact on public health, SARS-CoV-2 variants were labelled as "variants of concern" (*e.g.*,

Alpha, Beta, Gamma, Delta, and Omicron) and "variant of interest" (*e.g.*, Eta, Iota, Kappa, Lambda) based on their attributes[3,4].

Each of the variants penetrates the human cells by binding cell surface receptor, angiotensin converting enzyme (ACE) 2 *via* spike protein subunit 1 (S1), while spike protein subunit 2 (S2) permits entry of virus by enabling fusion of virus-envelope with host cell membrane. This virus-cell fusion is facilitated by S protein priming by host cell proteases viz transmembrane protease serine 2 at a cleavage site (S1/S2), which is a polybasic furin cleft[3,4]. Fusion of viral and cell membrane is followed by the entry of virus inside the host cell to release the genetic material *i.e.,* positive sense RNA. This RNA genome is the template for synthesis of new negative sense RNA with the help of RNA-dependent RNA polymerase. Newly synthesized RNA in turn facilitate in the synthesis of positive sense RNA, which is responsible for the production of new cytoplasmic proteins, namely nucleocapsid (N) protein and membrane (M) protein. N protein binds to freshly synthesized positive sense RNA and M protein facilitates its assimilation into the endoplasmic reticulum (ER) to form nucleocapsids. These nucleocapsids are finally transferred to the cell membrane *via* ER lumen and Golgi vesicle to the extracellular space *via* exocytosis[5,6].

These newly released virions infect the neighbouring healthy cells and manifest the COVID-19 with a diverse spectrum of symptoms, ranging from asymptomatic disease to severe symptoms, primarily associated with the respiratory system. However, emerging scientific evidence highlights the multisystemic nature of the disease *i.e.,* involving extrapulmonary clinical manifestations such as myocardial infarction, neurological, ocular, dermatologic, gastrointestinal, kidney failure and liver dysfunction, owing to the tropism of the virus for ACE 2, expressed in different human cells[7]. In fact, liver injury is the front and centre among the extrapulmonary manifestations and the most common pattern being mild-to-moderate hepatocellular injury; observed in 14%-53% of the hospitalized patients with COVID-19. Furthermore, epidemiological studies have revealed that over one-half of infected patients with SARS-CoV-2 had deranged liver function tests characterized by abnormal levels of hepatic-specific aminotransferases and other hepatic-specific biochemical indices[8,9], while a small subset of patients was found to be with acute

liver damage and fulminant hepatic failure[10,11]. Altered biochemical indices were more frequent in severely-ill COVID patients in contract to patients presenting with mild to moderate illness[12,13].

In addition, certain therapeutic compounds of various groups {antibiotics (azithromycin and ceftriaxone), antiviral [remdesivir (RDV), lopinavir (LPV)/ritonavir (RTV), favipiravir, umifenovir, and triazavirin], antimalarial [hydroxychloroquine (HCQ)], adjuncts, steroids (dexamethasone), immunomodulators [tocilizumab (TCZ)] and administration} of convalescent plasma from fully recovered COVID-19 patients as an investigational treatment or a higher dose of interleukin-1β inhibitor (anakinra)[14] administered to infected patients and for the management of infection-associated cytokine storm has a hepatotoxic potential in high doses[15,16] and was substantiated by the findings that toxicity got relieved after the cessation of these agents in *in-vitro/in-vivo* experiments[15,16]. Thus, insinuating drug induced liver injury (DILI) in the pathogenesis. Overall, available data suggest that the spectrum of hepatic damage in SARS-CoV-2 infection may be accredited to the direct cytopathic effect of the virus through the ACE2 receptor, indirect involvement by systemic immune-mediated inflammation and by iatrogenic causes *i.e.,* drug induced[17,18].

Moreover, underlying genetic factors could also contribute to COVID-19-related liver abnormalities, by reason of the fact that it occurs only in a subset of patient population. In accordance with this, a substantial number of genetic-based and or association studies have addressed the hosts' genetic makeup towards the predisposition to the development and progression of COVID-19-related liver injury, in an order to recognize the patient cohort for high clinical priority in terms of early or novel therapeutic interventions, albeit with equivocal results.

Since there are limited data on the individuals' genetic susceptibility to SARS-CoV-2 infection-related liver abnormalities, a detailed understanding of the influence of specific genotypes towards the same will be crucial for the clinical outcomes. In addition, a substantial evidences from the scientific literature pointed the degree of liver toxicity is owing to certain therapeutic regime employed in the treatment of infected with SARS-CoV-2. But, only a handful of researchers had

methodically and comprehensively explored the complete array of DILI in COVID-19 patients. Hence, it is worth reviewing the genetic and drug-induced perspectives on COVID-19-related liver injury. This review emphasizes on elucidating the DILI in COVID-19 patients along with a genetic insight for the development of SARS-CoV-2 infection related liver injury by providing the evidence from most current pertinent publications using relevant keywords from online databases.

MATERIALS AND METHODS

Procedure adopted for relevant literature search

Using various electronic databases, namely *Reference Citation Analysis* (*RCA*), PubMed China National Knowledge Infrastructure and Web of Science, our team carried the relevant literature search using the following MeSH keywords: DRUG INDUCED LIVER INJURY AND COVID-19 OR DRUG INDUCED LIVER INJURY AND SARS-COV-2 OR DRUG INDUCED LIVER INJURY AND 2019 nCOV OR DRUG INDUCED LIVER INJURY AND CORONAVIRUS DISEASE with regards to drug-induced perspectives and COVID-19 AND LIVER INJURY AND POLYMORPHISM OR SARS-COV-2 AND LIVER INJURY AND POLYMORPHISM OR COVID-19 AND LIVER INJURY AND GENETIC INSIGHT OR SARS-COV-2 AND LIVER INJURY AND GENETIC INSIGHT as for genetic insight of hepatic damage.

The criteria for inclusion were, original articles, case series or reports, brief communication or letters to the editor. However, article must be in English language and published during the period 1st December 2020 to 30th April 2022. All the initially included articles' references were further screened to add any plausible relevant literature. However, studies with animal or cellular models were not encompassed. Other criteria for exclusion were; injury due to SARS-CoV-2 infection itself, and hepatic injury from herbal or dietary supplements. Finally, after eliminating duplicate articles, thirty-one (2 and 29 relating to genetic insight and DILI respectively) out of 727 (14 and 713 articles respectively for genetic insight and DILI) research literatures were selected for review after removing the duplicate research literature.

Document retrieval

By means of aforementioned key words and in line with inclusion and exclusion criteria of the study, authors Sonagra AD, Dholariya S, Motiani A searched for research literature in the electronic databases. Authors who performed the literature search then shared the documents with all authors, and each one further reviewed articles to ensure the fulfilment of inclusion criteria. Thereafter Parchwani D and Singh R picked the articles to be finally included in the study. There authors then extracted the data viz, author, site and sample size of study, stages of COVID-19, severity of diseases, medication, outcome and or DILI. All the mentioned information were extracted by standardized data extraction tables in duplicate.

RESULTS

A total of 8 studies on genetic insight and 287 studies concerning DILI were retrieved after removing the duplicate or repeated publications. Among the included studies, in total 31 studies were considered suitable for the qualitative synthesis; comprising two and twenty-nine, in regards to genetic insight and DILI respectively. Extraction of research articles (Figure 1) were performed as per the guideline prescribed in PRISMA statement 2020 and was done in accord to published protocol (PROSPERO ID: CRD42022311838).

Hepatoxicity of commonly used drugs used in the treatment of SARS-CoV-2

Medications and or therapies employed in COVID-19 management, such as RDV, LPV/RTV, ribavirin, TCZ, hydroxyl chloroquine sulfate, *etc.* are potentially hepatotoxic, specifically in high doses[19] and its administration in the form of polypharmacy exponentially increased the risk of DILI. The hepatotoxicity information as described across the included studies are compiled herewith for most frequently used drugs in the therapeutic regime of COVID-19 and Table 1 depicts the relevant information as per study. The most frequently associated drugs with DILI were RDV, LPV/RTV, TCZ, HCQ (+/-) azithromycin, ceftriaxone, paracetamol, and enoxaparin.

RDV: A broad-spectrum nucleotide analogue prodrug, primarily used for hospitalized patients with COVID-19 is known to inhibit viral RNA polymerases. RDV had the maximum DILI rate/administration and screening of World Health Organization safety reports database revealed a total of three hundred and eighty-seven adverse drug reactions (ADRs) reports of RDV by late 2021. Out of which majority were hepatobiliary (61%), followed by 34% hepatic. The most common documented adverse liver outcome in different studies were elevated hepatic specific aminotransferase in the range of 15%-50%, hypoalbuminemia and hyperbilirubinemia[20]. Levels of aminotransferase elevation were more prominent in seriously ill patients, suggesting a possibility of occurrence of various adverse events due to severity and gravity of SARS-CoV-2 infection. Furthermore, studies also accounted that in a subset of patients RDV treatment was discontinued on account of the abnormally high liver aminotransferase levels[20]. Chew *et al*[21] conducted a study in a sample of 834 COVID-19 hospitalized patients and reported that 12.6% (*n* = 105) of patients showed a > 5 upper normal limit (UNL) of serum aspartate aminotransferase. Among the adverse lung events, TCZ & RDV were significantly associated with DILI on univariate analysis. Further, Delgado *et al*[22] conducted a retrospective observational study for the assessment of DILI by a pharmacovigilance program using laboratory signals. Out of 8719 patients admitted for COVID-19, 4.9% patients developed DILI. The drugs commonly associated with DILI were HCQ, azithromycin, TCZ and ceftriaxone. Out of these, RDV had the highest incidence rate of 992.7 DILI per 10000 defined daily doses.

These adverse events are further corroborated by individual case reports/series, for *e.g.*, in one such report[23], after two days administration of RDV, a sharp elevation in the level of alanine aminotransferase (ALT) was observed, which got instantly corrected after stopping RDV. Correspondingly, in other reported cases[24,25], increased levels of liver enzyme was found in patients on RDV +/- HCQ, which were initially medicated with LPV/RTV. In a case series reported in the United States, Carothers *et al*[26] suggested that administering acetylcysteine has a positive impact on overall health of patient and reversal of acute liver failure (ALF)

due to RDV where hepatic specific transaminase [ALT and aspartate aminotransferase (AST)] levels > 5000 IU/L, in addition to increased total bilirubin levels (3.1 mg/dL) serum ammonia (161 µmol/L) and international normalized ratio (INR) of 2.3.

LPV/RTV: Among the therapeutic regime of COVID-19, LPV/RTV were among the forerunners for contributing the liver ADRs. The same is apparent in a study (n = 217), that reported that LPV/RTV was found to be associated with 63% of total ADRs, while other drugs (umifenovir, chloroquine, and antibacterial drugs) combinedly make-up the 47% of ADRs[27]. Correspondingly, a study of 148 patients reported that 48% developed hepatic function abnormality after admission to the hospital[28]. They emphasized that among such patients, 57.8 % of the patients were on LPV/RTV (57.8%). In another study, authors reported an abnormal liver function by 12.1% with the addition of each collateral medication[29]. Moreover, combined use of LPV/RTV with arbidol (umifenovir) in patients who are not terminally ill are also found to have elevated risk of liver injury up to 3.58 times in comparison to those patient cohorts whose treatment regimen did not include aforesaid medications. Along these lines *i.e.*, drug-drug interactions (polypharmacy), it is postulated that RTV being an inhibitor of chromosome 3 gene cluster (C3)A4, could promote hepatic toxicity from azithromycin[26]. Similarly, metabolic interactions between the two medications [LPV/RTV and arbidol (umifenovir)] were studied *in-vitro* using human liver microsomes by Serviddio *et al*[30] and concluded that LPV/RTV significantly impedes the arbidol metabolism ($P < 0.005$), which may be the cause of DILI.

In a study with 163 mild and 29 severe patients with COVID-19, the multivariate analysis suggested RTV as one of the independent risk factors [odds ratio (OR) = 4.75, 95% confidence interval (CI): 1.89-16.55, $P < 0.001$] in COVID-19 patients with liver injury[31]. On the contrary to most of the studies which reported a moderate-to-severe elevations in serum aminotransferase levels in patients under LPV/RTV treatment, Cai *et al*[9], though, reported increased odds of liver injury by four-fold in LPV/RTV treated group among the enrolled 417 COVID-19 patients in China, but the most significant increase was gamma-glutamyl transferase activity and total

bilirubin. As the drug lacks efficacy, it was discontinued from the COVID-19 treatment regimen.

TCZ: TCZ, an interleukine-6 receptor antagonist monoclonal antibody, primarily used for severely ill COVID-19 patients to arrest the cytokine storm. Guaraldi *et al*[32] did not find any adverse effects on liver function test (LFT) in a retrospective study involving 1351 COVID-19 patients treated with TCZ. In the following line of evidence, Serviddio *et al*[30] published a case series from Italy, who displayed substantial altered hepatic and lung function tests after administration of LPV/RTV, HCQ, and azithromycin for five to seven days. These patients showed an improvement in both liver and lung function after the use of TCZ within 3 wk. However, the first case reported of DILI due to the use of TCZ, does not deny the possibility of serious hepatotoxicity when used with other hepatotoxic drugs. In this case, one day after TCZ administration, the levels of serum transaminase elevated up to 40-fold (AST of 1076 IU/L and ALT of 1541 IU/L)[33].

One more retrospective cohort study with 1827 patients, conveyed that a positive correlation exists with usage of RDV, LPV/RTV, HCQ, and TCZ resulting in hepatotoxicity[34]. They observed peak hospitalization liver transaminase elevations more than 5 times the UNL.

HCQ with or without azithromycin: Most cases of DILI were reported for HCQ after its emergency use authorization for COVID-19 infection. Kelly *et al*[35], conducted an analysis in two groups of 134 patients, one group was treated with HCQ/azithromycin, while other one was devoid of this targeted therapy. They reported no significant difference in the liver function tests between the two groups. On the contrary, a ten-fold elevation in levels of liver transaminases in serum after HCQ administration was reported by Falcão *et al*[36]. They revealed that serum levels of hepatic enzymes rapidly declined after the withdrawal of HCQ from the treatment regimen.

Corticosteroids: Systemic corticosteroids, mainly dexamethasone are widely used in patients with SARS-CoV-2 infections. However, on independent basis hepatoxicity is uncommon. Associated with minor, self-limiting elevations in serum aminotransferase. A study (*n* = 1040 COVID-19 patients) reported that the administration of corticosteroids was found to be correlated (adjusted OR = 3.9) with development of acute liver injury on independent basis (95%CI: 2.1-7.2)[16].

Enoxaparin: Associated with minor, self-limiting elevations in serum aminotransferase, but values > 5 UNL are uncommon. Sporadic cases of mild increases in serum bilirubin and alkaline phosphatase are reported.

Favipiravir: A single study testified the adverse liver event of favipiravir administration was found[37]. Authors of the study portrayed a COVID-19 patient who manifested a bacterial pneumonia as a complication of COVID-19 during his hospital stay. The therapeutic regime of the patient was LPV/RTV combined with interferon β-1b. Following administration of favipiravir, liver transaminases and total bilirubin elevated multifold suggesting a cholestatic liver injury. The liver injury, in this case, may have been triggered by antibacterial treatment, which may have been further deteriorated by giving high dose of favipiravir.

Tang *et al*[38] found 17.31% (*n* = 3425) of DILI in the cohort of 19782 COVID-19 patients. They observed reporting OR for DILI was 2.99 (2.593.46), 5.39 (4.63-6.26) and 3.16 (2.68-3.73) when comparing LPV-RTV with all other drugs, RDV and HCQ/chloroquine respectively. A single-centre, open-label, parallel-arm, stratified randomized controlled trial done by Panda *et al*[39] observed DILI in the form of elevated liver enzymes in 2 out of 67 participants who received a high dose of ribavirin.

Genetic insight towards SARS-CoV-2 induced liver injury

In the matter pertaining to a genetic propensity towards SARS-CoV-2 induced liver injury, in the United Kingdom Biobank cohort[40], an elevated risk score of genetic fatty liver disease (FLD) based on glucokinase regulator, MBOAT7, patatin like

phospholipase domain containing 3 (PNPLA3), and transmembrane 6 superfamily 2 human gene genetic variants have not been found to be associated with a higher probability of developing severe SARS-CoV-2. Hence, this finding challenges ahead against a causal role for metabolic associated FLD in COVID-19 and implies that genetic susceptibility to hepatic fat deposition does not, in and of itself, increase the risk of developing a severe form of the disease[40]. However, contrary to this, MBOAT7 rs641738[41], rs11385942 G>GA at C3 and rs657152 C>A at ABO blood locus were significantly associated with the severity of livery injury in admitted SARS-CoV-2 patients[42,43]. Thus, the genetic-basis of SARS-CoV-2 induced liver injury is not yet fully comprehended and additional research is required to validate the involvement of any specific variant form. Table 2 depicts the commonly employed therapeutic drugs for COVID-19, with its hepatic side effects and 'Likelihood Score' by the LiverTox database[44].

DISCUSSION

The primary analysis of this review revealed that DILI is due to the large-scale use of drugs/off-label drugs in the prophylactic and therapeutic regimen of COVID-19 and the causal relationship of genetic susceptibility with hepatic damage in SARS-CoV-2 infected patients is incomprehensible. Hepatic damage may arise either through intrinsic or idiosyncratic mechanisms. The intrinsic pathway is predictable and has a short latency period. However, COVID-19 related liver injury (drug induced and or genetic-based) follows predominantly the idiosyncratic mechanism *i.e.,* it is unpredictable with a variable latency period[19].

Drugs like LPV/RTV were associated with moderate to severe elevation (> 5 UNL) of hepatic specific aminotransferases in serum and exhibited a significantly (4 times) higher chances of liver injury[9]. The degree of hepatic damage varies in wide spectrum *i.e.,* from injury to hepatocytes to complete stagnation of bile acid secretion (cholestatic injury) or may be both in certain cases[44]. Correspondingly, medication of COVID-19 patients with LPV/RTV might exaggerate dysfunction of hepatic cells in particular to hepatitis B virus and hepatitis C virus infection cases[45]. However, the efficacy of LPV/RTV in SARS-CoV-2 infection patients is not fully understood

and requires further evaluation[46,47]. In contrast, a study in China pointed out administration of antibiotics, ribavirin, non-steroid anti-inflammatory drugs are not associated with statistically significant risk of hepatic damage[9].

Likewise, studies evaluating the ADR/ADE of RDV reported that it could lead to liver injury, barring one[48]. Liver injury caused by RDV manifested only after third day of its administration, as elevated hepatic-specific transaminases, coagulopathy, and hepatic encephalopathy. N-acetyl cysteine is recommended for the management of ALF induced by RDV and discontinuation of drug for progression to ALF[26]. It is suggested that following criteria should be considered for an immediate cessation of RDV treatment; elevation of ALT more than five-times of its UNL or elevation of alkaline phosphatase more than two-times of its UNL, and increased level of total bilirubin more than two-times of its UNL or immediate incidence of coagulopathy or in cases where patients condition is deteriorating[49]. Thus, to diminish RDV-instigated toxic effects, assessment of liver status must be done before drug initiation and continuous monitoring of LFTs should be done during the course of treatment.

However, the most contentious reports were of TCZ; a retrospective cohort study[32] as well as meta-analysis[46,47], reported that TCZ in itself is not associated with liver injury in COVID-19 patients, and even one study reported that TCZ had a positive effect on clinical and laboratory parameters, caused by the use of LPV/RTV[30], so much so that ALT levels fall within the normal range from > 5 times ULN after administration of TCZ. While on the other hand, a study conducted by Muhović *et al*[33] reported that hepatotoxic effects of TCZ is increased in cases where prior administration of antiviral drugs (LPV/RTV) has been done.

DILI in COVID-19 patients is often dependent on numerous factors, for instance, co-existing medical conditions (porphyria cutanea tarda, viral hepatitis, and rheumatologic diseases) could put the liver to an increased chance of developing toxicity due to recommended drugs[36]. Further, drug-drug interaction can lead to detrimental effects; viz chloroquine and its derivatives with anti-rejection immunosuppressants[50], the prevalence of liver damage was 15.2% in a sample size of 208 COVID-19 patients on RDV only, whereas it was 37.2% among 775 patients treated with RDV and LPV/RTV[51], substantiating the concept of polypharmacy.

Among included studies, one study reported that the grade of hepatotoxicity was not statistically different between the controls and cases, who were on HCQ and azithromycin[35]. Nevertheless, divergent findings are also frequently reported for HCQ, and has been hypothesized that the presence of pre-existing inflammation (mild to moderate), might increase the risk of liver damage by HCQ (+/- azithromycin) in the doses that are not hepatoxic, owing to production of cytotoxic metabolites from drug metabolism by inflammatory cells with the help of myeloperoxidase enzyme. Ivermectin (anti-parasite medication) and Colchicine (anti-inflammatory agent) drugs are well tolerated and have been reported to reduce the severity, period of hospital stay, and prevention of cytokine storm[46,52,53], but efficacy of these drugs in the management of SARS-CoV-2 infected patients is still not fully understood[54,55]. Hepatotoxic effects are not very well documented.

Mechanisms of DILI

Drugs employed in the management of SARS-CoV-2 infection, viz RDV, LPV/RTV, ribavirin, TCZ, HCQ or any other drugs are metabolized by the hepatic cells. Owing to this, liver damage with associated increased hepatic specific indices is predictable and same has been corroborated and cited in the scientific literature with various enlisted causes[56-60] (Table 3).

Certain critical biochemical properties of anti-COVID-19 drugs that might lead to hepatotoxicity in susceptible hosts are, lipophilicity, mitochondrial liability, generation of cytotoxic metabolites, their metabolic pathway in the liver, and the ability to inhibit hepatic transporters[61]. Patients who died with severe COVID-19 had moderate micro-vesicular steatosis, a condition characterized by a variant form of hepatic fat accumulation and modest lobular and portal activity in their liver biopsies, suggesting that the liver injury may have been due to either viral- or drug-induced. Steatosis in lieu of drugs occurs due to interference with β-oxidation of fatty acids, oxidative phosphorylation, or both by certain drugs[62], resulting in the accumulation of free fatty acids which in due course of time are converted to triglycerides[63].

Clinical and murine studies have provided evidence that certain pre-existing medical conditions, *e.g.,* inflammatory diseases, increased blood pressure and diabetes mellitus, augment the SARS-CoV-2 hepatic injury, possibly because of ACE inhibitors or angiotensin receptor blocker in these states, which result into ACE2 upregulation[64,65]. The presence of pre-existing non-alcoholic FLD sensitizes hepatocytes to antipyretic agents containing acetaminophen[66].

Reduced and or suppressed activity of CYPs family or cytochrome P450 (enzyme responsible for metabolism of xenobiotics) is also the plausible mechanism to alter the activity of liver cells[67]. CYPs are downregulated owing to repressive effects exerted by interleukins and cytokines, which are released in significant proportion during COVID-19 infection, leading to toxicity of several COVID-19 drugs[67]. Drug-drug interactions also play an important role in the development and progression of DILI, as exemplified by the clearance of umifenovir is compromised by concomitant use of LPV/RTV due to its inhibitory effect on cytochrome P3A[29].

A precedent of hepatic transporter inhibition by COVID-19 drugs to manifest the liver injury is reported by many studies with regard to LPV, a prominent blocker of multidrug resistance-associated protein-2 (MRP2). A study performed on rats[68], reported the accumulation of taurocholic acid inside the liver cells following the ten-minute exposure of rat liver cell to protease inhibitor (PIs) drugs, LPV and RTV, indicating that the PIs inhibits the efflux of bile salts from liver cells. Lending further support is by an experimental study to prove the inhibitory effect of PIs on MRP2[69,70] and by Holmstock *et al*[69], using 5(6)-carboxy-2′,7′-dichlorofluorescein through confocal imaging. Another recent study, done by Khalatbari *et al*[71] focused on oxidative stress damage leading to hepatotoxicity and FLD due to PIs, LPV and, RTV. They reported the interference of these drugs by ER-Golgi trafficking through inhibition of Ras converting CAAX endopeptidase-1 (RCE1) and any of its substrate, which in turn lead to development of fatty live and cellular stress.

Additionally, exhaustion of P450 activity to metabolize large and multiple amounts of COVID-19 drugs as a treatment regimen can also be the cause of hepatoxicity. Simultaneously, studies had reported that administration of certain drugs (LPV/RTV) assists in the reactivation of hepatitis B and C viruses and result in

hepatoxicity[72]; administration of HCQ in patients with porphyria cutanea tarda leads to significant liver damage[72], owing to communion of reactive metabolite of HCQ and inflammatory response due to SARS-CoV-2 infection[73].

However, there is a deficit of uniformity and standardization as to DILI, owing to lack of reliable and exclusive evidence pointing towards the drugs used in the treatment of COVID-19 disease, as the sole culprits. Moreover, there is considerable overlap and commonality in the presenting symptoms of hepatic damage due to COVID-19 infection per se and due to drugs given for its treatment. Increased vigilance on the part of the clinicians is warranted, so that cases of severe liver damage suspected to be caused by the drugs can be reported and entered into the National/International database. The R-value, can be considered as a diagnostic approach for the pattern of liver injury; *i.e.,* R > 5 to be considered as hepatocellular DILI, R < 2 as cholestatic DILI, and R value between 2 to 5 as mixed DILI (R-value = ALT value/UNL divided by ALP value/ULN).

Genetic aspect of hepatic consequences of SARS-CoV-2 infection

Irrespective of the above-mentioned drugs in the treatment regime, Machill *et al*[41], have reported that patients with carrier genotypes of MBOAT7 rs641738 polymorphism were found to have significantly elevated bilirubin, ALT, ALP levels and decreased serum albumin levels during hospitalization stay. Thus, pointing towards genetic susceptibility.

Dongiovanni *et al*[74] explored polygenic risk score of hepatic fat content (PRS-HFC) and genetic markers of liver fibrosis, viz PNPLA3 I148M variant; both PRS-HFC and PNPLA3 I148M are found to be inherited independently of dysmetabolism at conception to gain a better understanding into the relationship between FLD, liver damage, and COVID-19[23]. They reported that rs11385942 G>A at C3 and rs657152 C>A at ABO blood locus were significantly associated with the severity of liver injury in admitted SARS-CoV-2 patients[74]. In fact, although a greater ALT was related to a genetic propensity to FLDs during SARS-CoV-2, this was accompanied by reduced systemic inflammation or C-reactive protein levels and the maintenance of hepatic production or circulating serum albumin level in carriers with PNPLA3

I148M variant. The protective impact of the non-secretor ABO phenotype against SARS-CoV-2 infection has yet to be explained; it depends on whether or not differences in membrane glycan shedding underlie differential tissue susceptibility such as liver and lungs to infection. Finally, it was observed that the risk of severe COVID-19 in hospitalized patients was not observed to be elevated by the use of genetics-based assessment, which is a reliable unconfounded all-time proxy of a tendency to and progression of FLD. So, genetic propensity to obtain liver fat, despite aiding in liver injury, may unexpectedly defend against inflammation throughout SARS-CoV-2, suggesting that FLD predilection does not automatically lead to increased inflammation[42].

To summarize, with available evidence the present study outlines that the degree of lipophilicity of drugs, inflammatory response to the antivirals, metabolization by CYP3A4 in the liver, interference of drugs with various transporters in the liver, molecules/proteins accountable for protection against the xenobiotics, *e.g.*, organic anion transporting polypeptide 1B1, p-glycoprotein, MRP2, breast cancer resistance proteins and ER-Golgi trafficking primarily by inhibiting RCE1 are the underlying factors responsible for drug hepatotoxicity in SARS-CoV-2 infection treatment. Concurrently, drugs have a detrimental effect on bile salt export pump activity (an outflow transporter system, responsible for excretion of waste and foreign substances from the hepatic cells), and thus becomes a central factor in the cholestasis process. In addition, robust shreds of evidence are lacking regarding the genetic predisposition to hepatic dysfunction in SARS-CoV-2 infection. Larger prospective studies are warranted in this regard.

CONCLUSION

Hepatic dysfunction in SARS-CoV-2 infection could be the result of an individual drug or due to interactions among more than one drug and may be a subset of the patient population that had a genetic propensity. Thus, serial estimation of hepatic indices in SARS-CoV-2 infection hospitalized patients, especially patients on treatment with drugs, like RDV, LPV/RTV, favipiravir, HCQ and TCZ should be

done to take corrective actions on time for iatrogenic causes to avoid clinical deterioration.

Limitations

Nonetheless, our findings described here are only an assortment of evidence and do not imply causation. Other limitations include, firstly, the sample size as to the number of studies included for consideration was comparatively smaller. Secondly, methodology adopted in the included studied had a wide variation (as few studies only raised the probability of the DILI rather than confirming the role of drugs with certainty), therapeutic regimen, time duration difference in the sample collection after admitting in the ward, failure to record or variation in the time onset of disease/degree of liver injury and discrepancy in correcting different clinic-biochemical indices (viz sex, co-existing morbidities, age). Thirdly, discrepancy in the measurement of liver indices and sub-classification of the cases. Finally, only articles published in English were considered for analysis, it may have a local literature bias. In spite of all the aforementioned confines, present review brings an important systematic data on the genetic susceptibility of liver damage and DILI in SARS-CoV-2 infection.

ARTICLE HIGHLIGHTS

Research background

Available data advocate that the spectrum of hepatic damage in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection may be accredited to the direct cytopathic effect of the virus, indirect involvement by systemic immune-mediated inflammation and by iatrogenic causes *i.e.,* drug induced. Empirical use of potentially hepatotoxic drugs in the management of SARS-CoV-2 infection is considered as one of the major etiopathogenetic factor for the liver injury. Moreover, experimental and clinical evidences has shown that an underlying genetic factor may also concur. Hence, it is important to understand the host genetics and iatrogenic based mechanisms for liver dysfunction, so as to take timely remedial measures.

Research motivation

To identify drug induced liver injury in coronavirus disease 2019 (COVID-19) patients along with a genetic insight for the development of SARS-CoV-2 infection related liver injury, so as to provide better care and timely management of critical patients.

Research objectives

To explore drug induced and genetic perspective for the development of SARS-CoV-2 infection related liver injury.

Research methods

A systematic literature search was carried out in multiple electronic databases: PubMed, Reference Citation Analysis, China National Knowledge Infrastructure, and Goggle Scholar were explored by using related MeSH keywords and relevant data. The inclusion criteria were English language articles published between December 1, 2020 and April 30, 2022. All selected articles' reference list was further screened to identify additional possible research literature. There was no exclusion based on the study outcome and stage or severity of SARS-CoV-2 infection. However, studies with animal or cellular models were not encompassed. Other criteria for exclusion were; injury due to SARS-CoV-2 infection itself, and hepatic injury from herbal or dietary supplements. Finally, twenty-three [2 and 21 relating to genetic insight and drug induced liver injury (DILI) respectively] out of 727 (14 and 713 articles respectively for genetic insight and DILI) articles were selected for the review after removing the duplicate research literature.

Research results

The primary analysis of this review revealed that DILI is due to the large-scale use of drugs/off-label drugs in the prophylactic and therapeutic regimen of COVID-19 and the genetic susceptibility underlying liver damage in COVID-19 patients is not yet fully understood. COVID-19 related liver injury (drug induced and or genetic-based)

follows predominantly the idiosyncratic mechanism *i.e.*, it is unpredictable with a variable latency period. In most commonly used drugs, the hepatic specific aminotransferase and other biochemical indices were elevated and were significantly associated with severity, hospital stay, number of COVID-19 treatment drugs, and worse clinical consequences.

Research conclusions

Hepatic dysfunction in SARS-CoV-2 infection could be the result of individual drug or due to drug-drug interactions and may be a subset of patient population had a genetic propensity. Thus, serial estimation of hepatic indices in SARS-CoV-2 infection hospitalized patients should be done to take timely corrective actions for iatrogenic causes to avoid clinical deterioration.

Research perspectives

Additional prospective studies are warranted in this regard to justify DILI due to COVID-19 treatment regime along with the genetic predisposition of same which should provide optimization of disease status.

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