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Original Article

Longitudinal changes of liver function and hepatitis B reactivation in COVID-19 patients with pre-existing chronic hepatitis B virus infection

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Aim: With the current coronavirus disease (COVID-19) pandemic and high endemic levels of chronic hepatitis B virus (HBV) infection worldwide, it is urgent to investigate liver function changes of COVID-19 patients with chronic HBV infection, and how severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in turn affects the course of chronic HBV infection.

Method: We undertook a retrospective study based on 347 COVID-19 patients (21 vs. 326 with vs. without chronic HBV infection). With the propensity score matching (PSM) method, we yielded 20 and 51 matched patients for the HBV group and the non-HBV group, respectively.

Results: At the end of follow-up, all of these 71 patients achieved SARS-CoV-2 clearance (P=0.1). During the follow-up, 30% versus 31.4% in the HBV group versus non-HBV group progressed to severe COVID-19 (P=0.97). After PSM, the longitudinal changes of median values for liver biochemistries were not significantly different between the two groups. In the HBV group versus non-HBV group, 35% (7/20) versus 37.25% (19/51) (P=0.86) had abnormal alanine aminotransferase at least once during hospitalization, 30% (6/20) versus 31.37% (16/51) had abnormal aspartate aminotransferase (P=0.91), 40% (8/20) versus 37.25% (19/51) had abnormal γ-glutamyltransferase (P=0.83), and 45% (9/20) versus 39.22% (20/51) had abnormal total bilirubin levels (P=0.91). Moreover, three patients in the HBV group had hepatitis B reactivation.

Conclusions: Liver dysfunction presented in COVID-19 patients with/without chronic HBV. Moreover, those COVID-19 patients co-infected with chronic HBV could have a risk of hepatitis B reactivation. It is necessary to monitor liver function of COVID-19 patients, as well as HBV-DNA levels for those co-infected with HBV during the whole disease course.

Key words: COVID-19, HBV, hepatitis B reactivation, liver function

INTRODUCTION

CORONAVIRUS DISEASE 2019 (COVID-19), an emerging respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has recently become a pandemic. A total of 14348858 confirmed cases and 603691 deaths were reported globally as of 20 July 2020.[1] Unfortunately, neither targeted drugs nor vaccines are available to date, and the number of infections is growing around the world. For the foreseeable future, COVID-19 could constantly pose a great threat to the people of the world.

COVID-19 is typically characterized by the symptoms of viral pneumonia such as fever, fatigue, dry cough, and

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anosmia, which could evolve to respiratory failure. Unexpectedly, there is increasing evidence that some individuals with COVID-19 have frequent abnormal liver function.[2–4] It was observed that elevated liver biochemistries were more common in severe COVID-19 cases than in mild cases.[5] The limited autopsy results of liver of COVID-19 cases showed moderate microvascular steatosis,[6] or hepatocyte degeneration accompanied by lobular focal necrosis and neutrophil infiltration.[7] These histological characteristics of liver were not specific manifestations of liver damage caused by SARS-CoV-2. Therefore, it is currently unclear whether liver damage/dysfunction of COVID-19 patients is mainly due to the SARS-CoV-2 infection or other coexisting conditions.

Hepatitis B remains another major worldwide public health problem, with approximately 257 million individuals infected with hepatitis B virus (HBV), and more than 94 million suffer from chronic hepatitis B (CHB).[8] A large cohort study from China reported that 2.1% (21/1099) of enrolled COVID-19 cases had pre-existing hepatitis B.[9] Given the endemic and high burden of HBV infection, CHB could be one important comorbidity of pre-existing liver diseases affecting the outcome of COVID-19. It is confirmed that HBV infection can cause damage to innate immune responses and imbalance of adaptive immune responses.[10] Meanwhile, uncontrolled inflammatory innate responses and impaired adaptive immune responses causing by SARS-CoV-2 could lead to harmful tissue damage, both locally and systemically.[11] Therefore, co-infection of SARS-CoV-2 and HBV could increase the damage to immune function and liver. However, to the best of our knowledge, no studies have been carried out on the impact of chronic HBV infection on the disease progression and liver function changes of COVID-19 patients, and how the SARS-CoV-2 infection in turn affects the course of chronic HBV infection.

More evidence is urgently needed to guide the screening of HBV co-infection and management of comorbidity of CHB during the COVID-19 pandemic. In this study, we aimed to assess the independent effect of HBV infection on the outcomes of COVID-19 as well as the progression of HBV infection.

METHODS

Subjects

ALL DIAGNOSED COVID-19 patients according to WHO interim guidance, with or without chronic HBV infection, who were admitted to the Third People's Hospital of Shenzhen (China) between 1 January and 1 March 2020, were enrolled. Chronic HBV infection was determined on the basis of testing positive for hepatitis B surface antigen (HBsAg) and/or HBV-DNA at hospital admission and medical history of chronic HBV infection. The clinical outcomes of COVID-19 and dynamics of liver biochemistries were monitored up to 12 April 2020, the final date of follow-up. The inclusion criteria were as follows: (i) subjects diagnosed with COVID-19; (ii) records were well documented; and (iii) subjects with longitudinal follow-up, that is, liver function testing, chest computed tomography (CT) scan, or blood gas assay with at least across 2 days. The exclusion criteria were: (i) subjects without data available at baseline, that is, blood routine examinations, liver biochemistries, CT score,[12] and blood gas assay; (ii) subjects co-infected with HIV; and (iii) subjects co-infected with hepatitis virus other than HBV, or had liver diseases other than CHB. The process of patients' enrolment is presented in Figure S1.

The study protocol was consistent with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Board of the Third People's Hospital of Shenzhen (No. 2020-167). All subjects provided signed informed consent.

Clinical evaluation, follow-up, and outcomes

Baseline was defined as the first hospital admission due to COVID-19. At baseline and during follow-up, all subjects included in this study underwent routine examination, monitoring of liver biochemistries, and SARS-CoV-2 nucleic acid testing with a median follow-up interval of 3 days.

The primary outcome was progression to severe COVID-19, and the secondary outcomes included clearance of SARS-CoV-2, liver injury, and hepatitis B reactivation. The time point of COVID-19 onset was defined as the day of the presence of symptoms self-reported by patients who were further confirmed with SARS-CoV-2 after hospital admission. The virus clearance was defined by the presence of two consecutive negative results in quantitative polymerase chain reaction detection for SARS-CoV-2 RNA at an interval of 24 h, and the day of the first of these two tests was considered as the clearance day. Patients were discharged from hospital after the clearance of SARS-CoV-2. Hepatitis B reactivation was defined as the abrupt reappearance of HBV-DNA viremia in a patient with previously inactive or resolved HBV infection, or a sudden and rapid rise of HBV-DNA level by at least 2 $\log_{10}$ in those with previously detectable of HBV-DNA.[13] According to the national guidelines for community-acquired pneumonia, and the diagnosis and treatment plan for the new coronavirus in China, all COVID-19 patients were classified into

severe or mild cases based on chest radiography, clinical examinations, and symptoms.[14,15]

We analyzed the dynamics of liver biochemistry indicators (i.e. alanine aminotransferase [ALT], aspartate aminotransferase [AST], total bilirubin [TBIL], and γ-glutamyltransferase [GGT]) to investigate the liver function changes. The normal ranges of ALT, AST, GGT, and TBIL in this study were 0–45 U/L, 0–45 U/L, 0–49 U/L, and 1.7–21 μmol/L, respectively. As one patient co-infected with HBV underwent liver biopsy, we investigated the pathological characteristics of liver injury of this patient.

Statistical analysis

All statistical analyses were carried out using R 3.6.1. We undertook propensity score matching (PSM) on the selected 347 subjects so that the group co-infected with HBV was comparable to the group without HBV co-infection in terms of observed covariates at baseline.[16] The factors for propensity score calculation include age, gender, body mass index, time intervals between COVID-19 onset to hospital admission, number of comorbidities except for CHB, liver biochemistries (ALT, AST, GGT, and TBIL), PaO_2/FIO_2 ratio, chest CT score, CRP, lymphocyte count, and platelet count at baseline. The PSM process was carried out by using R package *MatchIt* with the nearest-neighbor method, 1:3 matching ratio, and a caliper size of 0.1. For baseline characteristics, we used median (interquartile range) for continuous variables and the Wilcoxon test for comparison; we reported count (percentage) for categorical variables and Fisher's exact test for comparison.

We used the Kaplan–Meier method to estimate cumulative probabilities for the clearance of SARS-CoV-2 and progression to severe COVID-19 and compared the probabilities between the HBV and non-HBV groups using a log–rank test. We used the multivariable Cox proportional hazards model to compare the risk of progression to severe COVID-19.

To investigate the longitudinal changes over time, we first undertook comparisons of the median values of liver biochemistries (ALT, AST, GGT, and TBIL) over time between groups using the Wilcoxon signed-rank test. Then we compared the values of these indicators between groups at each time point (to examine differences between groups) and compared the values of these indicators at each time point to their baseline values within each group (to examine differences within groups) using the Wilcoxon or Kruskal−Wallis test. In addition, we also reported the proportion of patients with abnormal values for liver biochemistries over time to examine the liver function changes, using the χ^2-test or Fisher's exact test to compare the proportions between groups.

All significance tests carried out were two-sided. *P*-values less than 0.05 were deemed statistically significant and 95% confidence intervals (CIs) were calculated for point estimates.

RESULTS

Baseline characteristics of COVID-19 patients before and after PSM

A TOTAL OF 347 COVID-19 patients with/without HBV co-infection (21 vs. 326) were analyzed before matching. In the HBV co-infection group, 20 were diagnosed as hepatitis B e antigen (HBeAg)-negative chronic HBV infection or HBeAg-negative CHB, and one patient had pre-existing cirrhosis not undergo any imaging examinations during the hospitalization for COVID-19. We describe the details of history of HBV infection and antiviral treatment and virological and serological testing at baseline in Table S1. The cirrhotic patient had been receiving tenofovir disoproxil fumarate treatment, which continued during hospitalization; none of the other patients had a history of antiviral treatment before hospital admission. The COVID-19 treatment drugs during hospitalization are shown in Table S2. The baseline characteristics of subjects before and after matching are summarized in Table 1.

The PSM of the entire study population yielded 20 and 51 matched patients for the HBV and non-HBV groups, respectively. The covariates used for matching and not used for matching were comparable between the two groups after matching (Table 1, all $P>0.1$).

No significant difference between HBV versus non-HBV groups in SARS-CoV-2 clearance or progression to severe COVID-19

At the end of follow-up, all patients in both groups achieved SARS-CoV-2 clearance and none of them died. The median time to SARS-CoV-2 clearance (21 days; 95% CI, 19–29 days) in the HBV group was longer than that in non-HBV group (14 days; 95% CI, 13–21 days), however, no significant difference was observed regarding the probability of SARS-CoV-2 clearance over time between the two groups ($P=0.1$, Fig. 1a).

During the follow-up period, 30% (6/20) and 31.4% (16/51) of patients in the HBV and non-HBV groups progressed to severe COVID-19, respectively; there was no difference between the two groups in the probability of progression to severe COVID-19 over time ($P=0.97$, Fig. 1b). By multivariate analysis, the risk of progression to severe COVID-19 was not statistically different between

Table 1 Baseline characteristics of COVID-19 patients before and after propensity score matching (PSM)

Variables	Before PSM			After PSM		
	Non-HBV (n=326)	HBV (n=21)	P-value	Non-HBV (n=51)	HBV (n=20)	P-value
Variables used for matching						
Age, years	47.00 (34.00, 60.00)	54.00 (42.00, 60.00)	0.164	56.00 (42.50, 64.00)	52.50 (41.00, 59.00)	0.344
Male sex	152 (46.6)	12 (57.1)	0.478	32 (62.7)	12 (60.0)	1.000
BMI, kg/m^2	23.20 (21.20, 25.60)	22.00 (20.60, 24.80)	0.238	22.00 (20.80, 24.20)	22.10 (20.60, 25.15)	0.964
Interval between onset to admission, days	3.00 (1.00, 6.00)	3.00 (2.00, 7.00)	0.656	3.00 (1.00, 5.50)	3.50 (2.00, 7.75)	0.279
Number of comorbidities			0.764			0.599
0	257 (78.8)	16 (76.2)		40 (78.4)	15 (75.0)	
1	48 (14.7)	3 (14.3)		9 (17.6)	3 (15.0)	
2	16 (4.9)	2 (9.5)		2 (3.9)	2 (10.0)	
3	5 (1.5)	0 (0.0)		0 (0.0)	0 (0.0)	
PaO_2/FIO_2 ratio	422.62 (367.92, 480.95)	434.29 (352.86, 472.86)	0.904	399.05 (337.86, 445.51)	427.86 (350.24, 479.64)	0.303
CT scan score	10.00 (4.00, 16.00)	8.00 (4.00, 16.00)	0.946	13.00 (5.00, 20.50)	9.00 (4.75, 16.50)	0.682
ALT, U/L	20.00 (15.00, 29.00)	31.80 (22.00, 39.40)	**0.002**	26.00 (16.00, 34.70)	30.40 (22.00, 36.85)	0.300
AST, U/L	26.00 (20.25, 34.53)	34.70 (27.00, 41.00)	**0.005**	29.80 (24.10, 37.30)	34.15 (27.00, 39.58)	0.303
TBIL, μmol/L	10.60 (8.20, 16.20)	13.60 (10.50, 16.10)	0.116	9.60 (8.25, 17.15)	12.60 (10.50, 16.43)	0.213
GGT, U/L	22.60 (15.72, 35.00)	30.00 (18.00, 44.70)	0.245	24.00 (16.30, 38.25)	28.50 (17.25, 43.42)	0.798
CRP, mg/L	8.70 (3.58, 24.67)	9.52 (2.05, 17.99)	0.872	14.60 (5.55, 27.51)	9.91 (4.26, 20.07)	0.331
eGFR, mL/min	105.32 (93.88, 116.49)	96.22 (92.46, 106.78)	**0.034**	98.33 (87.53, 107.09)	96.58 (92.28, 108.16)	0.788
Variables not used for matching						
Admission type			1.000			1.000
Non-severe	309 (94.79)	20 (95.24)		49 (96.08)	19 (95.0)	
Severe	17 (5.21)	1 (4.76)		2 (3.92)	1 (5.0)	
Type of comorbidity						
Diabetes	18 (5.5)	1 (4.8)	1.000	2 (3.9)	1 (5.0)	1.000
Hypertension	45 (13.8)	5 (23.8)	0.345	4 (7.8)	5 (25.0)	0.119
Cardiovascular disease	27 (8.3)	1 (4.8)	0.872	7 (13.7)	1 (5.0)	0.530
Cancer	5 (1.5)	0 (0.0)	1.000	0 (0.0)	0 (0.0)	NA
PLT, 10^9/L	187.00 (148.00, 230.75)	160.00 (140.00, 195.00)	0.055	163.00 (135.50, 200.00)	158.50 (138.50, 190.00)	0.701
FIB-4	1.35 (0.82, 2.34)	2.50 (1.18, 2.90)	**0.008**	1.86 (1.30, 2.65)	2.42 (1.17, 2.86)	0.641
APRI	0.32 (0.22, 0.47)	0.51 (0.32, 0.68)	**0.001**	0.41 (0.32, 0.60)	0.49 (0.31, 0.67)	0.420
Fibrinogen, g/L	3.86 (3.15, 4.58)	3.51 (2.90, 4.76)	0.411	4.00 (3.29, 4.80)	3.55 (2.90, 4.78)	0.378
Lymphocytes, 10^9/L	1.28 (0.99, 1.77)	1.22 (0.94, 1.64)	0.460	1.21 (1.02, 1.48)	1.21 (0.93, 1.62)	0.803
Neutrophils, 10^9/L	2.73 (2.00, 3.61)	2.33 (1.68, 3.30)	0.415	2.48 (1.92, 3.33)	2.40 (1.86, 3.54)	0.980
White blood cells, 10^9/L	4.61 (3.76, 5.78)	4.31 (3.48, 5.72)	0.407	4.27 (3.49, 5.25)	4.34 (3.48, 5.73)	0.863
Fever	222 (68.1)	12 (57.1)	0.425	36 (70.6)	12 (60.0)	0.565

(*Continues*)

Table 1. (Continued)

Variables	Before PSM			After PSM		
	Non-HBV (n=326)	HBV (n=21)	P-value	Non-HBV (n=51)	HBV (n=20)	P-value
Treatment						
Antiviral therapy	279 (85.58)	18 (85.71)	1.000	48 (94.12)	17 (85.00)	0.340
Methylprednisolone	80 (24.54)	6 (28.57)	0.678	16 (31.37)	6 (30.00)	0.910
NIV	44 (13.50)	4 (19.05)	0.510	12 (23.53)	4 (20.00)	1.000
IMV	14 (4.29)	1 (4.76)	1.000	2 (3.92)	1 (5.00)	1.000
ICU	28 (8.59)	2 (9.52)	0.701	9 (17.65)	2 (10.00)	0.717

Data are shown as number (%) or median (interquartile range).
P-values were calculated by the Mann–Whitney U-test or Fisher's exact test. P-values indicate differences between hepatitis B virus (HBV) and non-HBV group. $P<0.05$ was considered statistically significant.
ALT, alanine aminotransferase; APRI, AST to platelet ratio index; AST, aspartate transaminase; BMI, body mass index; CRP, C-reactive protein; CT, computed tomography; eGFR, estimate glomerular filtration rate; FIB-4, Fibrosis-4; GGT, γ-glutamyltranspeptidase; ICU, intensive care unit; IMV, invasive mechanical ventilation; NIV, non-invasive ventilation; PLT, platelets; TBIL, total bilirubin abnormal.

the two groups before (adjusted hazard ratio [HR] 0.75; 95% CI, 0.30–1.88; P=0.58) and after PSM (adjusted HR 1.39; 95% CI, 0.43–4.59; P=0.58) (Table S3).

Liver dysfunction in COVID-19 patients with and without HBV

In the HBV versus non-HBV groups, 35% (7/20) versus 37.25% (19/51) had abnormal ALT at least once during hospitalization, respectively (P=0.86). The proportion of abnormal ALT had a rise–fall trend in the HBV group but a constantly increasing trend in the non-HBV group following admission to hospital (Fig. 2a), which was 18.18% and 19.23% at 15 days in the two groups, respectively (P=0.94).

In the HBV versus non-HBV groups, 30% (6/20) versus 31.37% (16/51) had abnormal AST at least once during hospitalization, respectively (P=0.91). The proportion of abnormal AST had fluctuant reductions in both groups following admission to hospital (Fig. 2b), which declined to 0% and 7.69% at 15 days, respectively (P=1).

In the HBV versus non-HBV groups, 40% (8/20) versus 37.25% (19/51) had abnormal GGT at least once during hospitalization (P=0.83). The proportions of abnormal GGT remained high at 15 days in both groups (28% vs. 36.36%; P=0.70), despite a fluctuant reduction following admission to hospital (Fig. 2c). In these two groups, 45% (9/20) versus 39.22% (20/51) had abnormal TBIL at least once during hospitalization, respectively (P=0.91), however, all the patients in both groups had fluctuant reductions following admission to hospital and achieved the normalization of TBIL (Fig. 2d).

Longitudinal changes of liver biochemistries (ALT, AST, GGT, TBIL) in COVID-19 patients with versus without HBV

As the median of testing/assessing time intervals and follow-up durations were 3 days and 14 days, respectively, for liver biochemistries (ALT, AST, GGT, TBIL), we compared the dynamic levels of these indicators within/between the two groups at baseline and at 3, 6, 9, 12, and 15 days during hospitalization.

The median levels of liver biochemistries over time showed no significant difference between the two groups (Fig. 3; Wilcoxon signed-rank test: ALT, P=0.56; AST, P=0.58; GGT, P=0.43; TBIL, P=0.16). In addition, we found no significant difference in the ALT, AST, GGT, or TBIL levels between the two groups at each time point (Fig. S2).

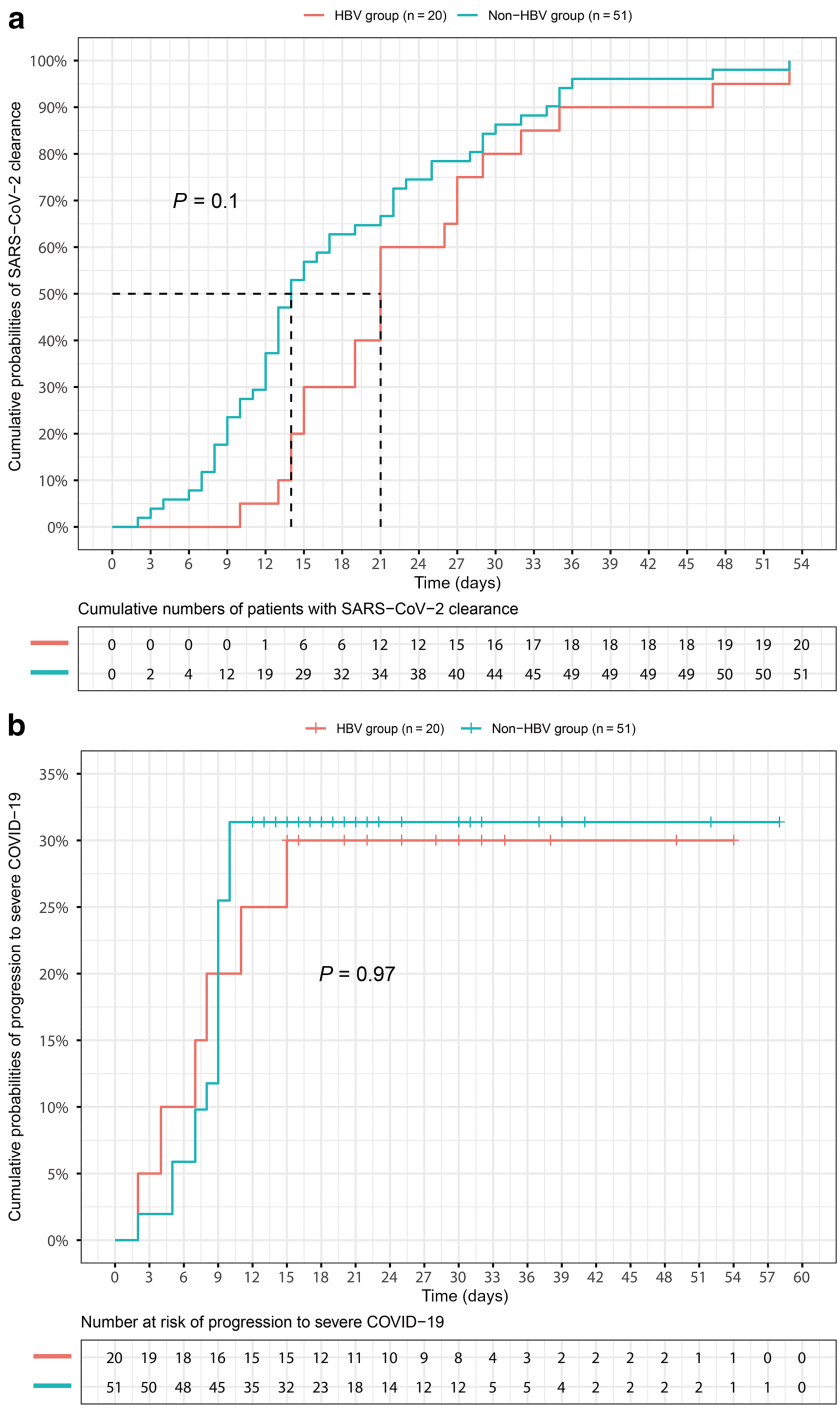

Figure 1 Cumulative probabilities of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) clearance and progression to severe status for COVID-19 patients with hepatitis B virus (HBV) versus without HBV infection. (a) Cumulative probabilities of SARS-CoV-2 clearance. (b) Cumulative probabilities of progression to severe COVID-19. [Color figure can be viewed at wileyonlinelibrary.com]

Hepatitis B reactivation found in three COVID-19 patients with chronic HBV infection

Of the 20 COVID-19 patients with chronic HBV infection, 19 had HBV-DNA viral load testing at least twice during hospitalization; one patient did not undergo HBV-DNA viral load testing. Of the 19 patients, three patients had HBV reactivation, 15 patients had the HBV-DNA viral loads maintained at low levels (<300 IU/mL) or undetectable, and one patient's HBV-DNA viral loads were at high levels throughout hospitalization.

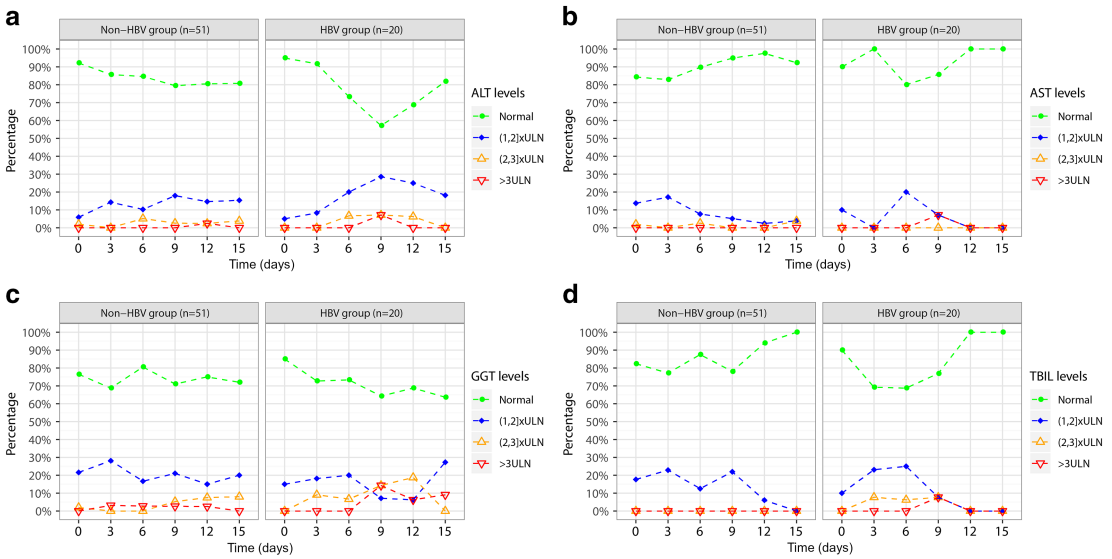

Figure 2 Proportion of patients with abnormal values of liver biochemistries over time in COVID-19 patients with hepatitis B virus (HBV) versus without HBV infection. (a) Alanine aminotransferase (ALT). (b) Aspartate aminotransferase (AST). (c) γ-Glutamyl transferase (GGT). (d) Total bilirubin (TBIL). [Color figure can be viewed at wileyonlinelibrary.com]

All three patients with hepatitis B reactivation were HBeAg-negative and did not received any antiviral treatment for HBV before admission. We further described the dynamics of HBV-DNA viral load and liver biochemistries and treatment information in Figure 4, as follows.

Case 1

Case 1 received methylprednisolone therapies on days 3–6 of admission, and interferon-α1b treatment (atomized inhalation) on days 5−9 of admission. Hepatitis B virus DNA viremia was undetectable at day 9, but was measured as 3.30 $\log_{10}$ IU/mL at day 30, and finally as 4.05 $\log_{10}$ IU/mL when discharged. The ALT and AST levels increased to 387 IU/L (8.6× upper limit of normal [ULN]) and 497 IU/L (11× ULN), respectively, on day 8 of admission.

Case 2

Case 2 received methylprednisolone therapy on days 2–5 of admission, and interferon-α1b treatment (atomized inhalation) on days 1–25 of admission. The HBV-DNA levels were at 3.5–5 $\log_{10}$ IU/mL during the early period of hospitalization but rapidly increased from 2.26 $\log_{10}$ IU/mL on day 29 to 6.65 $\log_{10}$ IU/mL on day 31. The ALT and AST levels were mildly high (<2× ULN) at admission and then restored to normal levels on day 3 of admission.

Case 3

Case 3 received interferon-α1b treatment (atomized inhalation) on days 1–53 of admission. Hepatitis B virus DNA viremia was undetectable at admission but hepatitis B reactivation (detectable as 1.30 $\log_{10}$ IU/mL) was found on day 9 of admission. Both ALT and AST were persistently normal during hospitalization.

Pathological characteristics of liver of one COVID-19 patient with CHB

One female patient who was 33 years old, and whose body mass index was 17.8 kg/m^2 at the time of admission to hospital, had a history of HBV infection for more than 20 years but had not received antiviral treatment for CHB previously. This patient had no history of alcohol use. The maximum levels of ALT and AST of this patient were 31.8 U/L and 35.6 U/L during hospitalization, respectively. The patient was discharged from hospital after SARS-CoV-2 clearance but was readmitted due to hepatalgia and had a liver biopsy 40 days after the clearance of SARS-CoV-2. Detailed information regarding her treatment course and laboratory tests is provided in Figure S3. The results of liver needle biopsy for this patient showed the structure of the hepatic lobules was clear, the hepatocytes in the liver plate were arranged orderly, with some diffuse swelling of hepatocytes (ballooning degeneration), and necrosis of isolated hepatocytes. No canalicular bile plugs or interface hepatitis were seen. Reticulin staining and Sirius Red staining

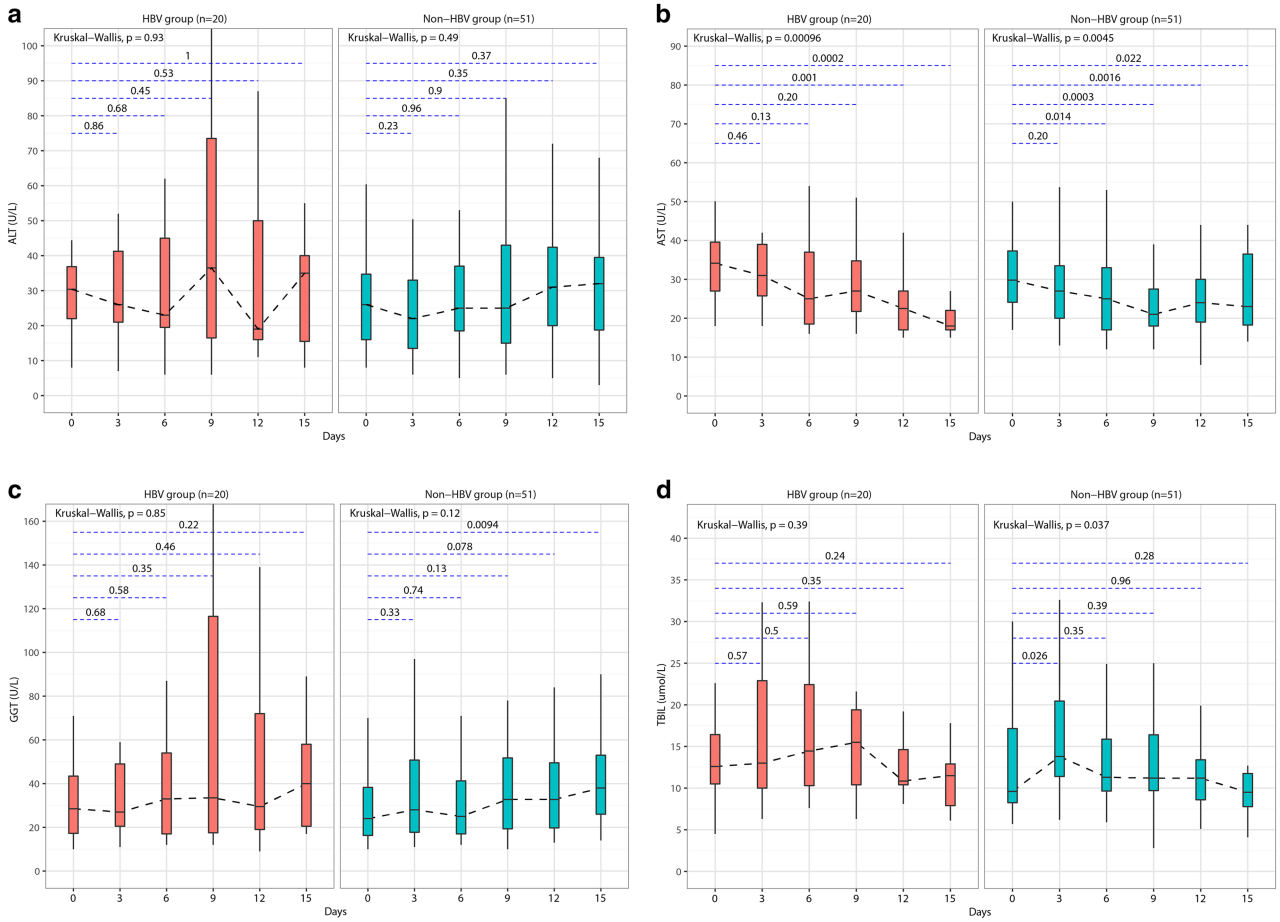

Figure 3 Longitudinal changes of liver biochemistries for COVID-19 patients with hepatitis B virus (HBV) versus without HBV infection. Alanine aminotransferase (ALT) (a), aspartate aminotransferase (AST) (b), γ-glutamyltransferase (GGT) (c), and total bilirubin (TBIL) (d) levels changed over time within each group. [Color figure can be viewed at wileyonlinelibrary.com]

indicated periportal fibrosis. The portal tracts were infiltrated with few inflammatory cells. The immunohistochemical analysis showed positive for HBsAg and negative for hepatitis B core antigen (Fig. 5).

DISCUSSION

THIS OBSERVATIONAL STUDY found no significant difference in probability of SARS-CoV-2 clearance and progression to severe COVID-19 over time in COVID-19 patients with versus without chronic HBV infection. We observed similar dynamics and non-significant differences at each time point on liver biochemistries (ALT, AST, GGT, and TBIL) between the two groups. However, we observed a continuous abnormality of ALT and GGT for both groups, which could be due to SARS-CoV-2 infection. More importantly, we identified three patients who underwent hepatitis B reactivation. This study provided preliminary evidence concerning the effect of chronic HBV infection on the outcomes and liver function of COVID-19 patients and added important data to support the management of COVID-19 patients with chronic HBV infection for physicians.

Investigation of SARS-CoV-2 shedding will be the key for determining the risk of transmission and formulating the criteria for releasing from quarantine. For the non-HBV COVID-19 patients, we found the median time of SARS-CoV-2 clearance was 12 days after the onset of COVID-19 symptoms, which was consistent with a study on eight discharged COVID-19 patients from Singapore (median, 14 days).[17] Despite the confirmed host immune dysfunction resulting from chronic HBV infection, our results reveal that chronic HBV infection could not delay the SARS-CoV-2 shedding for COVID-19 patients,

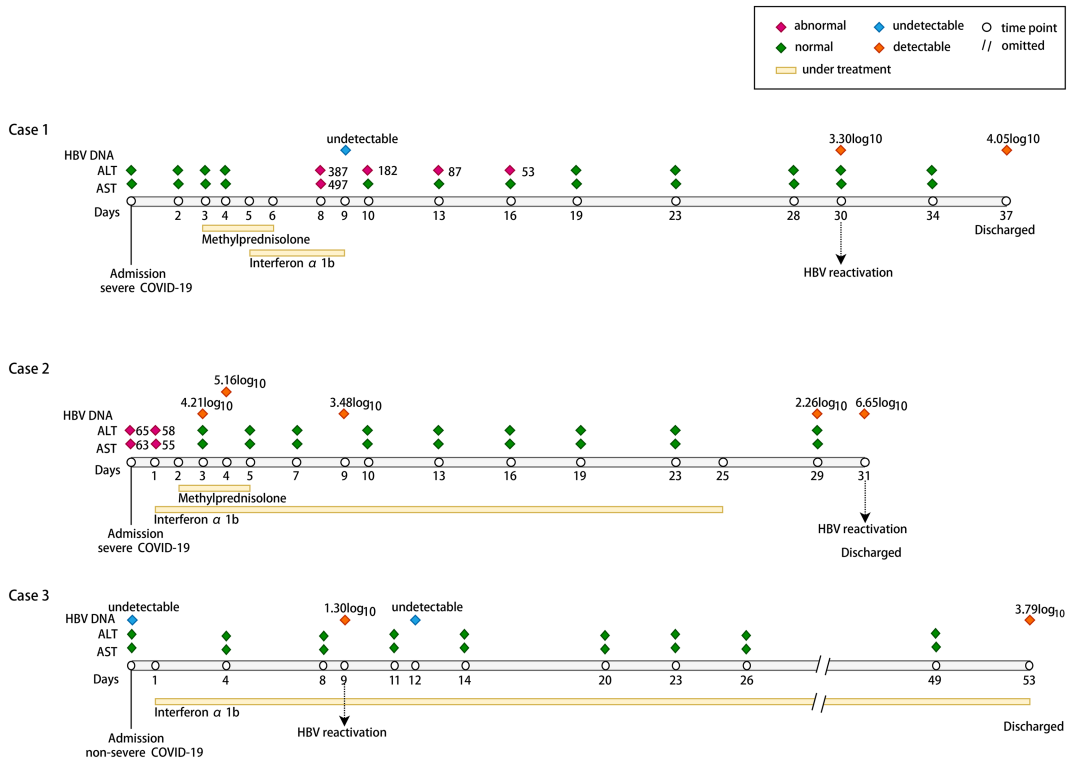

Figure 4 Hepatitis B virus (HBV)-DNA viral load, liver biochemistries, and treatment information of three COVID-19 patients with hepatitis B reactivation. ALT, alanine aminotransferase; AST, aspartate aminotransferase. [Color figure can be viewed at wileyonlinelibrary.com]

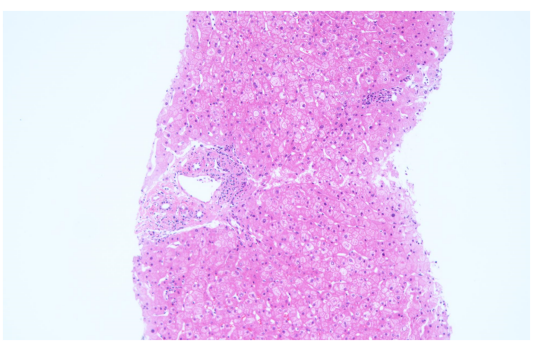

Figure 5 Pathological characteristics of liver of one COVID-19 patient who had chronic hepatitis B virus infection. [Color figure can be viewed at wileyonlinelibrary.com]

compared to those without chronic HBV. After excluding non-HBV-related chronic liver diseases, we explored the independent impact of chronic HBV infection on the progression to severe COVID-19 and found that chronic HBV infection did not increase the risk of progression to severe COVID-19. Together with these comparisons, we tend to conclude that the comorbidity of chronic HBV infection would not increase the risk of poor outcomes related to SARS-CoV-2. It is worth mentioning that only one patient in the HBV group was cirrhotic in this study. Patients with HBV-related cirrhosis typically have poor immune function compared to those who had chronic HBV infection but without cirrhosis. Therefore, further studies are necessary to examine the impact of HBV-related cirrhosis on the outcomes of COVID-19.

Previous studies have found that both hepatocytes and bile duct epithelial cells might also express the angiotensin-converting enzyme 2 receptor, and the latter has a higher concentration. It suggests SARS-CoV-2 might damage both hepatocytes and bile duct epithelial cells. Current studies showed that 6.2%–36.6% of patients had increased serum AST levels, and 21.3%–28.1% had elevated serum ALT levels.[18] A few studies reported abnormal proportions of TBIL (4.9%–10.53%)[4,19,20] and GGT (6.5%–14.8%)[4,19] at baseline. Our study also identified the abnormality of the above-mentioned liver laboratory tests. Moreover, with further analysis of longitudinal patterns, we found that the abnormality of AST and TBIL

manifested as transient elevation, and high proportions of patients with abnormal ALT and GGT did not achieve normalization of these two indicators. The dynamics of ALT and GGT suggested that SARS-CoV-2 possibly caused a continuous damage of bile duct epithelial cells and hepatocytes during the disease course. Although chronic HBV infection would not increase the injury of liver compared to non-HBV COVID-19 patients as suggested in this study, liver function monitoring is still essential for both COVID-19 patients with and without chronic HBV infection during the whole disease course.

Glucocorticoids have powerful anti-inflammatory effects, and have been confirmed to alleviate clinical symptoms, shorten treatment course, and improve the absorption of lung infiltrates for SARS patients.[21,22] In our study, six COVID-19 patients with HBV received methylprednisolone, one type of corticosteroid. It is well known that moderate to high doses (≥10 mg) of methylprednisolone can lead to a high risk of hepatitis B reactivation.[23,24] In our study, two of the three patients developed hepatitis B reactivation, which was possibly caused by methylprednisolone. However, one of the three patients who did not receive any corticosteroid also developed hepatitis B reactivation. Our results suggested that for COVID-19 patients who had chronic HBV infection, whether or not corticosteroids were used, they could have a risk of hepatitis B reactivation. Therefore, it is necessary to monitor the HBV-DNA levels of these patients, and physicians should take precautions against hepatitis B reactivation.

This study is not without limitations. The number of patients in the HBV group was small, although we expected to increase the test efficiency using PSM designed with a 1:3 matching ratio, and we validated our results by the multivariable Cox proportional hazards model. Additionally, we were unable to explore whether liver abnormality was associated with COVID-19 treatment drugs, as all the 71 patients in both groups had received them (Table S2).

In conclusion, this longitudinal study revealed that SARS-CoV-2 infection could possibly cause continuous abnormalities of ALT and GGT for COVID-19 patients with and without chronic HBV infection. Moreover, those COVID-19 patients co-infected with chronic HBV could possibly experience hepatitis B reactivation. The results in this study imply that it is necessary to monitor the liver function of COVID-19 patients, as well as the HBV-DNA levels for those with HBV during the whole disease course.

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SUPPORTING INFORMATION

ADDITIONAL SUPPORTING INFORMATION may be found online in the Supporting Information section at the end of the article.

Table S1. Details of history of hepatitis B virus (HBV) infection, antiviral treatment, and virological and serological testing at baseline in COVID-19 patients with chronic HBV infection.
Table S2. Summary of treatment information for 71 COVID-19 patients with/without chronic hepatitis B virus (HBV) infection.
Table S3. Multivariate analysis to compare the risk of progression to severe COVID-19.
Figure S1 Patient flow diagram.
Figure S2. Comparison of liver biochemistries between COVID-19 patients with and without hepatitis B virus (HBV) infection at each time point. (a) Alanine aminotransferase (ALT) levels. (b) Aspartate aminotransferase (AST) levels. (c) γ-Glutamyltransferase (GGT) levels. (d) Total bilirubin (TBIL) levels.
Figure S3. Disease course and liver biochemistries of one COVID-19 patient who had chronic hepatitis B virus (HBV) infection. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; TBIL, total bilirubin.