Severe Vitamin D Deficiency Is Strongly Associated with Liver Dysfunction and Disease Severity in Hepatitis B Virus Related Cirrhosis and Liver Failure Patients

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Summary Vitamin D deficiency is common in chronic liver diseases, it may increase the severity of the diseases. However, studies on the potential role of vitamin D in hepatitis B virus (HBV) related end stage liver disease are still limited. This study was performed to assess the prevalence of vitamin D deficiency in patients with different stages of chronic HBV infection and explore whether vitamin D deficiency can be established as an index of severity and prognosticator of disease progression. Serum 25(OH)D₃ levels were measured in a cohort of 363 patients with chronic HBV infection. Biochemical parameters, the alpha fetoprotein level, HBV DNA load, hepatitis B surface antigen level, and hepatitis B early antigen level were tested. The mean 25(OH)D₃ level was significantly lower in patients with chronic HBV-related liver disease than in healthy controls. Overall, 74.9% of patients had 25(OH)D₃ deficiency. The 25(OH)D₃ level was significantly positively correlated with the albumin level. In total, 77.6% (121/156) of patients with cirrhosis had vitamin D deficiency. The 25(OH)D₃ level significantly decreased as the Child–Pugh classification increased in severity. The 25(OH)D₃ level was negatively correlated with the model for end-stage liver disease (MELD) score and low 25(OH)D₃ concentrations were significantly associated with high MELD score and mortality in patients with liver failure. Vitamin D deficiency is prevalent in patients with chronic HBV infection. Severe vitamin D deficiency is strongly related to liver dysfunction and disease severity in the cirrhosis and liver failure patients.

Key Words vitamin D, vitamin D deficiency, 25(OH)D₃, hepatitis B virus, chronic liver disease

Vitamin D is widely known to have multiple extra-skeletal health functions and responsible for regulating metabolism, homeostasis along with cell proliferation, cell differentiation, immunomodulatory and anti-inflammatory effects. Since the liver is the main organ for vitamin D metabolism, vitamin D deficiency has been constantly reported in many causes of chronic liver disease, such as chronic hepatitis C (1–3), primary biliary cholangitis (PBC) (4), autoimmune hepatitis (AIH) (5) and nonalcoholic fatty liver disease (NAFLD) (6), advanced cirrhosis (7) and hepatocellular carcinoma (HCC) (8).

Vitamin D deficiency is also observed in the majority of hepatitis B virus (HBV)-infected patients. Most studies have shown that low serum levels of vitamin D are associated with high levels of HBV replication in patients with chronic hepatitis B (CHB) and contribute to poor clinical courses (9–12). However, Zhao et al. (13) and Yu et al. (14) failed to find an association between the serum HBV-DNA level and vitamin D con-

centration. Several studies have focused on the relationship between the vitamin D level and liver function. The vitamin D level is positively correlated with the albumin (ALB) level and platelet count and inversely correlated with the alanine transaminase (ALT) level during the active phase of hepatitis B (15–17). A vitamin D serum level of <10 ng/mL can be predictive of a low serum ALB level and more severe chronic liver disease (16, 17). However, other studies have shown that vitamin D insufficiency is not correlated with liver function parameters. Therefore, the association of vitamin D with the HBV replication status and liver function requires further study.

To clarify this issue, we investigated the serum vitamin D levels in a wider disease spectrum of chronic HBV-related liver diseases including CHB, HBV-related cirrhosis with different Child–Pugh categories, HCC, and liver failure to determine whether the serum vitamin D level can act as an indicator associated with the severity of HBV-related liver diseases.

METHODS

Patients. In total, 363 patients with chronic HBV infection and 80 healthy controls were enrolled in this

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Table 1. Association of serum 25(OH)D₃ levels with clinical and biological variables in HBV-infected patients.

	25(OH)D ₃ levels		p value
	<10 ng/mL (n= 110)	≥10 ng/mL (n=253)	
Female, %	41.8	17.3	0.006
Age, y	50.21±1.13	47.08±0.74	0.021
Blood sampling during summer/autumn, %	43.6/56.4	57.3/42.7	0.022
ALT, U/L	89.42±24.27	175.47±27.46	0.055
AST, U/L	87.23±16.30	139.72±19.65	0.099
ALP, U/L	123.07±8.91	130.23±6.35	0.526
GGT, U/L	95.10±15.99	106.67±8.64	0.492
ALB, g/L	34.05±0.68	37.92±0.38	<0.001
TBIL, μmol/L	76.62±11.68	72.71±7.74	0.781
WBC, ×10 ⁹ /L	4.49±0.29	5.05±0.19	0.109
PLT, ×10 ⁹ /L	98.16±6.10	116.34±4.22	0.017
PT, s	18.72±0.70	16.58±0.32	0.002
INR	2.12±0.18	1.93±0.23	0.501
Child–Pugh classification			
A/B/C, %	34.5/23.6/41.8	56.5/26.9/16.6	<0.001
MELD score	20.52±1.90	20.70±1.09	0.928
Disease state, %			0.001
CHB	14.5	85.5	
Compensated cirrhosis	21.4	78.6	
Decompensated cirrhosis	44.2	55.8	
HCC	32.5	67.5	
Liver failure, %	36.4	63.6	

ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; GGT, gamma glutamate transferase; ALB, albumin; TBIL, total bilirubin; WBC, white blood cell; PLT, platelet; PT, prothrombin time; INR, international normalized ratio; MELD score, the model for end-stage liver disease; CHB, chronic hepatitis B; HCC, hepatocellular carcinoma.

cross-sectional study at the First Affiliated Hospital of Xi'an Jiaotong University from 2015 to 2017. The patients were divided into five different clinical subgroups based on their clinical manifestations: CHB ($n=69$), compensated liver cirrhosis without HCC ($n=70$), decompensated liver cirrhosis without HCC ($n=86$), HCC with decompensated liver cirrhosis ($n=83$), and acute-on-chronic liver failure ($n=55$). The inclusion criterion for the present study was chronic infection with HBV, defined as a detectable hepatitis B surface antigen (HBsAg) level for ≥ 6 mo or intermittent elevation in the ALT level before antiviral therapy using nucleos(t)ide analogs or interferon. The exclusion criteria were other causes of liver disease or mixed causes (alcoholic hepatitis, hepatitis C, hepatitis D, autoimmune liver disease, Wilson's disease, or hemochromatosis); human immunodeficiency virus co-infection; renal dysfunction or malignant or severe chronic disease; treatment with vitamin D or calcium supplements, bisphosphonates, calcitonin, or hormone replacement therapy; and pregnancy or lactation. Decompensated cirrhosis is marked by the development of overt clinical signs, the most frequent of which are ascites, bleeding, encephalopathy, and jaundice (18). Acute-on-chronic liver failure is a syndrome characterised by acute and severe hepatic abnormalities resulting from different types of insults, in patients with underlying chronic

liver disease or cirrhosis but, in contrast to decompensated cirrhosis, has a high short-term mortality, mimicking the prognosis of acute liver failure (19).

The study was conducted according to the principles of the Helsinki Declaration, and written informed consent was obtained from all patients. As a part of the project A1463-080, the study was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University.

Quantification of 25(OH)D₃ serum levels. Fasting blood samples were obtained from all patients, and the serum was stored at -80°C for subsequent use. The 25(OH)D₃ level was measured using an automated electrochemiluminescence-based assay (Roche Diagnostics, Mannheim, Germany). The data are expressed in nanograms per milliliter (ng/mL). Based on the recommendation of the Endocrine Society and previous research (20), we classified the 25(OH)D₃ status as normal (≥ 30 ng/mL), insufficient (20–30 ng/mL), deficient (10–20 ng/mL), or severely deficient (<10 ng/mL).

Measurement of viral markers and biochemical parameters. The serum HBV-DNA level was quantified using the Roche COBAS TaqMan (Roche Diagnostics) with a lower limit of detection of 20 IU/mL. The serum HBsAg titer was determined with the Architect HBsAg quantitation assay (Abbott Laboratories, Chicago, IL, USA), based on an automated chemiluminescent microparti-

cle immunoassay performed in accordance with the manufacturer's instructions. The quantitative HBsAg level was reported in international units (IU)/mL. Samples with a higher HBsAg titer required further dilution to remain within the calibration curve. Serum hepatitis B early antigen (HBeAg) and the HBeAg antibody were measured using commercially available immunoassays (Abbott Laboratories).

In addition to epidemiological findings, the following biological parameters were measured in all patients to assess liver function: ALT, aspartate transaminase, alkaline phosphatase, γ -glutamyl transpeptidase, total bilirubin, direct bilirubin, ALB, prothrombin time, alpha fetoprotein (AFP), and platelet count. The degree of liver dysfunction was estimated by the Child–Pugh (CP) classification (21) and the model for end-stage liver disease (MELD) score (22). The patients were also divided into two groups according to their MELD score: 0 to 20 and >20 (23).

Statistical analysis. Statistical analysis was performed using the statistical package SPSS version 18.0 (SPSS, Inc., Chicago, IL, USA). Continuous variables are presented as mean $\pm$ standard deviation and quartile, and categorical variables are presented as frequency with percentage. HBV-DNA and HBsAg levels were transferred to log₁₀ IU/mL. The chi-square test was used for categorical variables. After the univariate analysis, only variables related to dependent variable were included in the multivariate logistic analysis for significant associations. The correlation between two continuous variables was analyzed using Spearman's bivariate correlation. In all analyses, a p value of <0.05 was considered statistically significant.

RESULTS

Association of serum 25(OH)D₃ levels with clinical and biological variables in HBV-infected patients

The mean serum 25(OH)D₃ level was 14.25 ± 7.09 ng/mL among all patients with chronic HBV-related liver disease, which was significantly lower than that in healthy controls (23.60 ± 6.20 ng/mL, $p < 0.05$). Overall, 74.9% (272/363) patients had vitamin D deficiency (<20 ng/mL). Clinical and biochemical characteristics according to 25(OH)D₃ levels (<10 ng/mL vs. ≥ 10 ng/mL) are summarized in Table 1.

Serum 25(OH)D₃ levels in HBV infected patients

Among all patients with chronic HBV infection, the mean 25(OH)D₃ level and percentage of vitamin D deficiency were 17.10 ± 6.16 ng/mL (60.9%) in patients with CHB, 17.16 ± 7.55 ng/mL (64.3%) in patients with compensated liver cirrhosis, 11.20 ± 5.99 ng/mL (88.4%) in patients with decompensated liver cirrhosis, 14.07 ± 7.34 ng/mL (83.1%) in patients with HCC, and 12.00 ± 6.07 ng/mL (72.7%) in patients with liver failure (Fig. 1). The 25(OH)D₃ levels were higher in patients with CHB than in those with decompensated liver cirrhosis ($p < 0.01$), HCC ($p = 0.006$), and liver failure ($p = 0.007$).

Factors associated with 25(OH)D₃ level

We performed a correlation analysis to further investigate the relationship between the serum 25(OH)D₃ concentration and different liver biomarkers. The 25(OH)D₃ level was significantly positively correlated with the ALB level ($r = 0.341$, $p < 0.001$) (Fig. 2A) but significantly negatively correlated with the prothrombin time ($r = -0.300$, $p < 0.001$) (Fig. 2B). However, the 25(OH)D₃ level was not significantly correlated with

Fig. 1. The mean value of serum 25(OH)D₃ level in different disease states. CHB, chronic hepatitis B; HCC, hepatocellular carcinoma.

Fig. 2. Correlation between serum 25(OH)D₃ level with ALB (A) and PT (B). ALB, albumin; PT, prothrombin time.

Fig. 3. Serum 25(OH)D₃ levels (A) and percentage of serum 25(OH)D₃ < 10 ng/mL according to Child-Pugh classification in cirrhosis patients.

Table 2. Univariate and multivariable analysis of risk of Child-Pugh classification in cirrhosis patients.

Variables	Univariate analysis			Multivariate analysis		
	OR	95%CI	p value	OR	95%CI	p value
Gender	1.829	0.855–3.912	0.120	1.341	0.556–3.235	0.514
HBV family history	0.505	0.232–1.097	0.084	0.582	0.250–1.357	0.210
Age	2.005	0.951–4.229	0.068	1.542	0.660–3.600	0.317
25(OH)D ₃ (ng/mL) (≥10 vs. <10)	3.568	1.658–7.678	0.001	3.654	1.558–8.572	0.003
Ig HBV DNA (IU/mL)	2.434	1.145–5.173	0.021	2.205	0.916–5.305	0.078
ALT (U/L)	1.081	0.327–3.578	0.899	1.236	0.308–4.970	0.765
FIB-4	1.700	0.525–5.500	0.376	4.721	0.526–42.343	0.166

HBV, hepatitis B virus; ALT, alanine transaminase; FIB-4, fibrosis 4 score.

HBV DNA ($r=0.075$, $p=0.153$), HBsAg ($r=0.055$, $p=0.297$), HBeAg ($r=0.007$, $p=0.893$), or AFP ($r=0.097$, $p=0.087$).

25(OH)D₃ status associated with Child-Pugh classification in cirrhosis patients

Of the 156 patients with HBV-related cirrhosis, 77.6% (121/156) had vitamin D deficiency. 25(OH)D₃ level significantly decreased as the Child-Pugh classification increased in severity: class A, 16.70 ± 7.12 ng/mL; class B, 12.76 ± 6.83 ng/mL; and class C, 10.01 ± 6.34 ng/mL ($p < 0.001$) (Fig. 3A). The percentage of 25(OH)D₃ level < 10 ng/mL was 43.2% among patients with Child-Pugh class C, 36.7% among those with Child-Pugh class B, and 20.0% among those with Child-Pugh class A (Fig. 3B). Low 25(OH)D₃ levels were significantly associated with high Child-Pugh classification (odds ratio [OR]=3.654, 95% confidence interval [CI]=1.558–8.572, $p=0.003$, Table 2) independently of other classical risk factors (age, sex, HBV DNA, ALT, fibrosis-4 score (FIB-4), HBV family history).

25(OH)D₃ status associated with MELD score and mortality in liver failure patients

Vitamin D deficiency was found in 72.7% of patients with liver failure. The 25(OH)D₃ levels were significantly higher in the patients with a MELD score of 0 to 20 than in those with a MELD score of >20 (15.0 ± 7.10 vs. 9.84 ± 4.11 ng/mL, respectively; $p=0.001$). In correlation analyses, 25(OH)D₃ levels were negatively asso-

Fig. 4. Correlation between serum 25(OH)D₃ level and MELD score in liver failure patients. MELD score, the model for end-stage liver disease.

ciated with the MELD score ($r = -0.360$, $p = 0.007$) (Fig. 4). Low 25(OH)D₃ concentrations were significantly associated with MELD score (odds ratio [OR]=4.119, 95% confidence interval [CI]=1.116–14.553, $p = 0.028$, Table 3) independently of other classical risk factors (age, sex, HBV DNA, ALT and HBV family history). 12 of 55 (21.8%) liver failure patients died during 3 mo of follow-up. Serum 25(OH)D₃ levels in the survival group were significantly higher than those in the death group (12.98 ± 6.25 ng/mL vs. 8.47 ± 3.76 ng/mL, $p = 0.021$). The percentage of serum 25(OH)D₃ levels < 10 ng/mL in the survival group were significantly

Table 3. Univariate and multivariable analysis of risk of MELD score in liver failure patients.

Variables	Univariate analysis			Multivariate analysis		
	OR	95%CI	<i>p</i> value	OR	95%CI	<i>p</i> value
Gender	0.433	0.126–1.487	0.184	0.299	0.067–1.334	0.114
HBV family history	0.610	0.236–1.581	0.309	0.648	0.221–1.901	0.429
Age	1.859	0.494–6.995	0.359	1.924	0.424–8.718	0.396
25(OH)D ₃ (ng/mL) (≥10 vs. <10)	4.141	1.288–13.314	0.017	4.119	1.166–14.553	0.028
Ig HBV DNA (IU/mL)	1.261	0.360–4.410	0.717	1.283	0.298–5.525	0.738
ALT (U/L)	1.700	0.525–5.500	0.376	1.113	0.265–4.676	0.883

HBV, hepatitis B virus; ALT, alanine transaminase.

Table 4. Univariate and multivariable analysis of risk of mortality in liver failure patients.

Variables	Univariate analysis			Multivariate analysis		
	OR	95%CI	<i>p</i> value	OR	95%CI	<i>p</i> value
Gender	1.650	0.410–6.646	0.481	1.451	0.262–8.034	0.670
HBV family history	0.795	0.249–2.543	0.669	1.308	0.303–5.646	0.719
Age	1.100	0.249–4.861	0.900	0.776	0.138–4.378	0.774
25(OH)D ₃ (ng/mL) (≥10 vs. <10)	5.167	1.309–20.390	0.019	4.925	1.009–24.037	0.049
Ig HBV DNA (IU/mL)	0.909	0.206–4.017	0.900	1.135	0.202–6.359	0.886
ALT (U/L)	0.338	0.066–1.739	0.194	0.364	0.055–2.420	0.296
MELD score	1.583	0.413–6.063	0.502	2.831	0.486–16.495	0.247
ALB (g/L)	0.435	0.114–1.663	0.224	0.506	0.096–2.666	0.422

HBV, hepatitis B virus; ALT, alanine transaminase; MELD score, the model for end-stage liver disease; ALB, albumin.

higher than those in the death group (66.7% vs. 27.9%, $p=0.014$). Severe vitamin D deficiency (<10 ng/mL) were significantly associated with the mortality in liver failure patients (odds ratio [OR]=4.925, 95% confidence interval [CI]=1.009–24.037, $p=0.049$, Table 4) independently of other classical risk factors (age, sex, HBV DNA, ALT, ALB, HBV family history and MELD score).

DISCUSSION

Previous studies have shown that vitamin D is related to chronic liver disease. Up to 93% of such patients have vitamin D insufficiency, and nearly one-third of them show severe vitamin D deficiency (24–26). A gradient change in the serum vitamin D level of patients with CHB according to latitude was observed from southern China (35.95 ± 11.10 ng/mL) to central China (29.06 ± 10.58 ng/mL) and northern China (24.44 ± 9.62 ng/mL) (10). The present study showed that vitamin D deficiency was common in patients with chronic HBV infection, but the serum vitamin D level was significantly lower than that reported in the above-mentioned study (10). The patients in the present study were from Shaanxi province, northwest China (31°–39°N), which is farther from the equator than southern China (23°–28°N) and central China (29°–36°N). Thus, we conclude that the different vitamin D levels were greatly

influenced by geographic latitude. However, this cannot explain why the vitamin D levels were lower than in northern China (37°–43°N). The difference may be related to other causes of vitamin D deficiency, such as sex, season, exposure to sunlight, obesity, disease spectrum, or genetic variation in vitamin D metabolism (27–30). However, further analysis could not be carried out because of the lack of relevant data.

In this study, we found a positive correlation between the serum vitamin D level and ALB level in patients with chronic HBV infection, too. The serum ALB level is a recognized indicator of liver synthesis ability, indicating that the serum vitamin D level is related to liver synthesis function. A better liver synthesis ability is associated with a higher serum vitamin D level (31). However, no significant correlation of the serum vitamin D level with HBV DNA quantification, HBsAg quantification, or HBeAg quantification were found in this study, which are consistent with the results reported by Zhao et al. (13) and Yu et al. (14). In contrast, other studies (10–12) have shown that lower vitamin D levels are associated with higher HBV-DNA levels in HBV-infected patients. In those studies, patients with cirrhosis only accounted for a small percentage of the study population. This discrepancy may be explained mainly by the presence of patients at different disease stages with different degrees of liver synthetic function. Therefore, the

association between the vitamin D level and the HBV replication status and its mechanism needs to be further studied.

The association of vitamin D with liver cirrhosis has been more intensively discussed in chronic hepatitis C (6) and in NAFLD (32) patients, rather than in chronic hepatitis B. Of the 156 HBV-infected patients with cirrhosis, we found that the mean 25(OH)D₃ levels was lower in patients with Child–Pugh C than Child–Pugh A or B, low 25(OH)D₃ levels were significantly associated with Child–Pugh classification. It suggests a correlation between vitamin D and the severity of cirrhosis. Patients with cirrhosis often have impaired liver function, malabsorption and dietary lacks, which can lead to vitamin D deficiency. By interacting with vitamin D receptor, vitamin D can prevent oxidative stress, affect the migration, proliferation and gene expression of fibroblasts, and reduce inflammation and fibroblast activity of hepatic stellate cells (33–36). Therefore, decreased 25(OH)D₃ level might favor the progression of fibrosis. The vitamin D-binding protein (DBP), is the main transporter for 25(OH)D₃ in the circulation, transporting approximately 85% of total 25(OH)D₃. The liver is one of the organs that are responsible for producing DBP. All liver diseases with reduced synthetic liver function are associated with a reduction in circulating DBP, which will lead to a decrease in serum total vitamin D concentration, while serum free vitamin D often remains within the normal range (37). However, Lai et al (38) found that free 25(OH)D₃ in cirrhosis with low serum albumin level was lower than cirrhosis with normal serum albumin level, suggesting that serum free 25(OH)D₃ was different in patients with liver diseases at different stages. The specific relationship between the disease progression in liver diseases and serum free 25(OH)D₃ still needs further study.

In patients with liver failure, 25(OH)D₃ level was negatively correlated with the MELD score, and severe vitamin D deficiency (<10 ng/mL) were significantly associated with MELD score and the mortality, too. Consistent with previous studies in alcoholic liver disease (ALD) (39, 40). The results in the present study suggested a significant association of vitamin D with the degree of liver dysfunction and severe vitamin D deficiency (<10 ng/mL) may predict high mortality in HBV-infected liver failure patients.

The present study has some limitations. First, it was performed in only one center. Second, we did not observe the effects of vitamin D supplementation on the progression of chronic HBV infection. We plan to conduct a prospective study to investigate these issues.

In conclusion, vitamin D deficiency is prevalent in patients with chronic HBV infection, especially in patients with decompensated cirrhosis, HCC, and liver failure. Severe vitamin D deficiency is strongly related to liver dysfunction and disease severity in the cirrhosis and liver failure patients.

Authorship

Data collection and manuscript writing: SML and

WW; statistical analysis of the data: LS, XLY and YRC; interpretation of the data: XJL, JZL and FY; research conception and design: XCA and XZ.

Disclosure of state of COI

We confirm that the manuscript has been read and approved by all named authors. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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