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Original paper

Association of serum 25-hydroxyvitamin D_3 levels and insulin resistance with viral load and degree of liver fibrosis in Egyptian chronic HBV patients: a case-control study

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Abstract

Aim of the study: To assess serum 25-hydroxyvitamin D_3 level and insulin resistance (IR) in hepatitis B virus (HBV) patients compared with controls and to evaluate the correlation with HBV viral load, severity of liver disease and degree of liver fibrosis.

Material and methods: A case-control study. Sixty HBV patients and 60 controls were enrolled. Chemiluminescence was used to determine 25-hydroxyvitamin D_3 levels. Insulin resistance was evaluated using the homeostasis model assessment method. Polymerase chain reaction was used to quantify HBV viral loads. Severity of liver disease was assessed by Child-Pugh scores. Transient elastography was used to evaluate the degree of liver fibrosis.

Results: 25-Hydroxyvitamin D_3 deficiency is more prevalent among HBV patients compared to controls. 25-Hydroxyvitamin D_3 levels declined considerably as viral load rose ($p < 0.001$). 25-Hydroxyvitamin D_3 level declined as liver fibrosis progressed (34.0 ±0.0 ng/ml in F1 vs. 12.67 ±8.0 ng/ml in F4) and the severity of the disease increased (22.75 ±6.36 ng/ml in Child A vs. 5.50 ±0.58 ng/ml in Child C). Insulin resistance is more prevalent among HBV patients compared to controls and it appeared to deteriorate progressively with boosting of the viral load, degree of fibrosis and severity of liver disease ($p < 0.001$).

Conclusions: HBV patients had significantly lower 25-hydroxyvitamin D_3 levels compared to healthy individuals and HBV infection is associated with IR. 25-Hydroxyvitamin D_3 deficiency and IR were associated with HBV viral loads, severity of liver disease, and degree of liver fibrosis.

Key words: liver fibrosis, viral load, serum 25-hydroxyvitamin D_3 levels, hepatitis B virus (HBV) infection, insulin resistance (HOMA-IR).

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Introduction

Hepatitis B virus (HBV) infection is a worldwide public health issue. Acute/fulminant hepatitis or various forms of chronic infection are clinical manifestations of HBV infection. HBV infection can result in the inactive carrier state, chronic hepatitis, liver cirrhosis, or hepatocellular carcinoma (HCC) [1]. Around one-third of the world's population has been infected, and over 350 million people suffer chronic HBV infection.

Gender, age, immunological response, HBV DNA levels, and other variables determine the outcome of infection [2]. HBV patients are at an increased risk of cirrhosis and HCC, which can be the cause of death in almost half of the cases [3].

25-Hydroxyvitamin D_3 is a fat-soluble vitamin that regulates calcium, phosphorus, and bone mineral homeostasis by acting through vitamin D receptors in a variety of tissues. It also has non-calcaemic effects in the body, including immunomodulatory, anti-inflammatory, and anti-fibrotic effects. Low 25-hydroxyvitamin D_3 levels have been linked to autoimmune illness, infectious disease, and the development of cancer in clinical studies. Although 25-hydroxyvitamin D_3 has been shown to be a key immunological modulator in the treatment of hepatitis C virus infection and metabolic liver disease, the link between 25-hydroxyvitamin D_3 levels, HBV viral load, and liver damage in HBV-infected individuals is largely unexplored [1]. Under normal physiological conditions, the precursor of vitamin D is hydroxylated to its major circulating form, 25-hydroxyvitamin D_3, in the liver. Thus, the liver plays a crucial role in vitamin D activation. In the liver, cytochrome P450 (CYP) isoforms microsomal CYP2R1 and mitochondrial CYP27A1 mediate this conversion process. As a result, liver dysfunction might be one probable cause of 25-hydroxyvitamin D_3 insufficiency or deficiency [4]. On the other hand, 25-hydroxyvitamin D_3 concentrations have been linked to the course and prognosis of liver disease in previous literature [3].

25-Hydroxyvitamin D_3 has a crucial role in fibrosis progression and the inflammatory response. 25-Hydroxyvitamin D_3 has been shown to inhibit collagen expression and profibrotic factors and to have immunomodulatory effects on immune cells, especially T lymphocytes. 25-Hydroxyvitamin D_3 has antifibrotic effects on hepatic stellate cells. Since the key factor of chronic liver disease prognosis is liver fibrosis, identifying the degree of fibrosis is crucial for determining the prognosis and developing a treatment approach in patients with chronic liver disease [5].

Insulin resistance (IR) is thought to be caused by a lack of glucose metabolic capability, resulting in the need for more insulin to elicit the same physiological response. Hyperinsulinaemia is the principal pathophysiology associated with metabolic syndrome, and it can cause a wide range of abnormalities in blood vessels, kidneys, and muscles. Diabetes mellitus and metabolic syndrome are also risk factors for atherosclerosis. Because metabolic syndrome is now recognized as a severe risk factor for cardiovascular disease, it is critical to treat it with preventive and comprehensive management. As a result, early detection of high-risk groups is crucial to successful health promotion. HBV infection appears to promote the emergence of both IR and diabetic mellitus. According to experimental studies, hepatitis B x protein (HBx) disrupts the insulin signalling system and thus HBV infection is associated with IR [6]. Because of its association with steatosis development, fibrosis progression, and non-response to treatment, IR has been considered as a significant risk factor in patients with chronic hepatitis C. The impact of HBV infection on human insulin sensitivity, on the other hand, has not been thoroughly investigated.

The aim of this study is to assess serum 25-hydroxyvitamin D_3 level and IR in HBV patients compared with healthy controls as well as to evaluate the possible correlation with HBV viral load, the severity of liver disease, and the degree of liver fibrosis.

Material and methods

A case-control study. Ethical approval was obtained from the institutional ethics committee (approval number: 21-514) and all patients and controls who participated in the study provided their informed consent. The study was conducted in compliance with the Declaration of Helsinki's ethics standards, as well as good clinical practice recommendations and local regulatory requirements. A total of 60 individuals with HBV, aged 18 years and up, presenting to the Hepatology Clinic at Alexandria Main University Hospital, were enrolled in the study. Cirrhosis, liver cancer, hepatitis C, clinical evidence of hepatic decompensation (hepatic encephalopathy, ascites, variceal bleeding), diabetes, other malignancies, other chronic illnesses, infection, pregnancy, taking medications known to affect 25-hydroxyvitamin D_3 metabolism such as vitamin or mineral supplements (calcium, vitamin D_3, or hormonal therapy) or regular use of medication(s) known to impair glucose tolerance or insulin secretion, abnormal thyroid functions, history of gastrectomy or chronic pancreatitis, prior antiviral therapy, abnormal renal function, and systemic immune diseases were among the exclusion criteria. Demographic data including age and gender were collected. A Beckman UniCel DxC800 Synchron (Analyzer) was used to perform liver function testing, which included measuring alanine transaminase (ALT) and aspartate transaminase (AST) enzyme levels (Beckman Coulter, California, USA). A chemiluminescence test was used to determine serum 25-hydroxyvitamin D_3 levels. A deficiency was defined as a serum 25-hydroxyvitamin D_3 concentration of less than 20 ng/ml, insufficiency as a value of more than 20 ng/ml and less than 30 ng/ml,

and normal as a concentration of more than 30 ng/ml [1]. IR was evaluated using the homeostasis model assessment (HOMA) method. HOMA-IR is calculated as follows: fasting blood glucose (mg/dl) × fasting insulin/405 [1].

The 7500 Fast Real-Time PCR System was used to quantify HBV viral loads using real-time polymerase chain reaction (RT-PCR) (Applied Biosystems, California, USA). The quantitation detection limit was set at 20 IU/ml. HBV patients were categorized according to the severity of liver disease into Child-Pugh A, B, and C groups according to their Child-Pugh scores [7].

A transient elastography device (FibroScan; Echosens, Paris, France) was used to evaluate liver stiffness as an indication of liver fibrosis. The units of measurement are kilopascals (kPa). The interquartile range (IQR) was established as the index of the intrinsic variability of liver stiffness values corresponding to the interval of valid measurements between the 25th and 75th percentiles comprising 50% of the valid data. Liver stiffness values with 10 validated measurements and an IQR-to-median-value ratio (IQR/M) of less than 0.3 were considered reliable. The fibrosis stage was determined by the transient elastography liver stiffness score: nil or minimum fibrosis (equivalent to F0-F1) less than 7.1 kPa; moderate fibrosis (F2), 7.1 to 9.4 kPa; severe fibrosis (F3), ≥ 9.5 kPa. A fibrosis score of ≥ 14.6 kPa was considered as advanced liver fibrosis (F4) [5, 8].

During the same period of time, 60 healthy age- and sex-matched control subjects were recruited to avoid the effect of seasonal variation on serum 25-hydroxyvitamin D_3. Serum 25-hydroxyvitamin D_3 level, HOMA-IR, and degree of liver fibrosis were evaluated in the control group for standardization and comparison.

Statistical analysis

Data analysis was done using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp.). Categorical data were represented as frequency and percentages. The χ^2 test was applied to investigate the association between the categorical variables. Alternatively, the Monte Carlo correction test was applied when the expected cell counts were less than 5. For continuous data, they were tested for normality by the Kolmogorov-Smirnov test. Normally distributed data were expressed as mean and standard deviation. Student's *t*-test was used to compare two groups for normally distributed quantitative variables. On the other hand, the Mann-Whitney test was used to compare two groups for non-normally distributed quantitative variables while the Kruskal-Wallis test was used to compare different groups for non-normally distributed quantitative variables. The significance of the obtained results was judged at the 5% level.

Results

HBV patients were matched to the control group regarding their age and gender distribution with an average age of 46.88 ±5.29 years and a male predominance.

The liver function tests as reported by AST and ALT did not show a significant difference between HBV cases and control; however, the degree of hepatic fibrosis among the HBV cases was more severe in comparison to the controls. More than 56% of the control group had a slight degree of liver fibrosis (F1), while HBV cases showed various degrees of fibrosis where the mainstream was F3 (48.3%) and F4 (30.0%). The majority of HBV cases were classified as Child A (73.3%) and only 6.7% were graded as Child C. Additionally, the viral load was categorized as low in 75% of the HBV group and 18.3% had a high viral load (Table 1).

The distribution of serum 25-hydroxyvitamin D_3 level among cases and controls was comparable as only 10% of HBV cases and 18% of controls were considered as having a normal serum 25-hydroxyvitamin D_3 level. Nevertheless, the average level of serum 25-hydroxyvitamin D_3 among the HBV cases was significantly lower than that of controls (19.02 ±8.42 vs. 22.43 ±8.96 ng/ml respectively) (Table 1).

Insulin resistance, as measured by HOMA-IR, was significantly more evident among HBV cases (4.98 ±1.82) compared to the controls (1.51 ±0.66) (Table 1).

In Table 2, the relations between serum 25-hydroxyvitamin D_3 and HOMA-IR with viral load, the severity of liver disease, and degree of liver fibrosis are presented. Serum 25-hydroxyvitamin D_3 levels declined considerably as viral load rose, reaching their lowest point (6.91 ±1.45 ng/ml) among patients with the greatest viral load ($p < 0.001$). Similarly, the level of serum 25-hydroxyvitamin D_3 declined as liver fibrosis progressed and the severity of the disease increased; it dropped from 34.0 ±0.0 ng/ml among patients with the least fibrosis (F1) to 12.67 ±8.0 ng/ml among those with the most severe fibrosis (F4), $p < 0.001$. Likewise, the same clinical finding was confirmed in the Child-Pugh group, with the lowest serum 25-hydroxyvitamin D_3 level of 5.50 ±0.58 ng/ml in the Child C group vs. 22.75 ±6.36 ng/ml in Child A patients. Remarkably, there appears to be a causal relationship between serum 25-hydroxyvitamin D_3 levels and viral load as well as the degree of liver disease as the values of serum 25-hydroxyvitamin D_3 trended upward with the decreasing

Table 1. Comparison between the two studied groups according to laboratory investigations and degree of fibrosis

Variable	Cases (*n* = 60)	Control (*n* = 60)	*P*-value
Age (years)			
Mean ±SD	46.88 ±5.29	48.35 ±5.73	0.148
Median (min.-max.)	48.0 (34.0-61.0)	47.0 (38.0-64.0)	
Sex, *n* (%)			
Male	44 (73.3)	34 (56.7)	0.056
Female	16 (26.7)	26 (43.3)	
AST			
Mean ±SD	24.93 ±3.52	25.52 ±3.67	0.376
Median (min.-max.)	25.0 (18.0-33.0)	25.0 (17.0-35.0)	
ALT			
Mean ±SD	29.10 ±10.21	25.13 ±3.30	0.147
Median (min.-max.)	26.50 (14.0-49.0)	25.0 (19.0-36.0)	
Serum 25-hydroxyvitamin D_3 (ng/ml), *n* (%)			
Deficiency (< 20)	30 (50.0)	20 (33.3)	0.139
Insufficiency (20-30)	24 (40.0)	29 (48.3)	
Normal (> 30)	6 (10.0)	11 (18.3)	
Mean ±SD	19.02 ±8.42	22.43 ±8.96	0.031*
Median (min.-max.)	19.0 (5.0-36.0)	24.50 (5.0-38.0)	
HOMA-IR			
Mean ±SD	4.98 ±1.82	1.51 ±0.66	< 0.001*
Median (min.-max.)	5.10 (0.90-8.60)	1.45 (0.60-2.80)	
Degree of fibrosis, *n* (%)			
F0	1 (1.7)	25 (41.7)	< 0.001*
F1	2 (3.3)	34 (56.7)	
F2	10 (16.7)	1 (1.7)	
F3	29 (48.3)	0 (0.0)	
F4	18 (30.0)	0 (0.0)	
Severity of liver disease, *n* (%)			
Child A	44 (73.3)		
Child B	12 (20.0)		
Child C	4 (6.7)		
Viral load, *n* (%)			
High	11 (18.3)		
Moderate	4 (6.7)		
Low	45 (75.0)		

*SD – standard deviation, t – Student t-test, U – Mann-Whitney test, χ^2 – chi square test, p – p value for comparing between the studied groups, *statistically significant at p ⩽ 0.05*

Table 2. Relation between serum 25-hydroxyvitamin D_3 levels and HOMA-IR with viral load, degree of fibrosis and severity of liver disease in case group (*n* = 60)

Variable	*n*	Serum 25-hydroxyvitamin D_3 (ng/ml) Mean ±SD	HOMA-IR Mean ±SD	*P*-value
Viral load				
Low	45	22.56 ±6.42	4.22 ±1.28	
Moderate	4	12.50 ±0.58	6.08 ±1.69	< 0.001*
High	11	6.91 ±1.45	7.68 ±0.61	
Degree of fibrosis				
F0	1	36.0#	0.90#	
F1	2	34.0 ±0.0	1.75 ±0.92	< 0.001*
F2	10	27.50 ±3.81	2.98 ±0.57	
F3	29	18.41 ±5.24	4.78 ±0.66	
F4	18	12.67 ±8.0	6.99 ±1.30	
Severity of liver disease				
Child A	44	22.75 ±6.36	4.20 ±1.29	< 0.001*
Child B	12	9.83 ±2.79	6.96 ±1.37	
Child C	4	5.50 ±0.58	7.65 ±0.66	

#number of cases (n) = 1

severity of the liver disease and lowering of the viral load.

Meanwhile, IR, as measured by HOMA-IR level, appeared to deteriorate progressively with boosting of the viral load, degree of fibrosis, and severity of liver disease ($p < 0.001$). The lowest HOMA-IR was reported among those with low viral load (4.22 ±1.28), those with F1 fibrosis (1.75 ±0.92), and among the Child A group (4.20 ±1.29). Additionally, the HOMA-IR values surged significantly with escalating viral load and worsening of liver condition.

Discussion

This study evaluated the variations in serum 25-hydroxyvitamin D_3 levels between patients with HBV and healthy controls, as well as the relationship between serum 25-hydroxyvitamin D_3 levels, IR, and HBV viral load, degree of severity of liver disease, and the degree of liver fibrosis. Our study's major findings are considerably lower serum 25-hydroxyvitamin D_3 levels in HBV patients as compared to healthy controls, and an inverse relationship between serum 25-hydroxyvitamin D_3 levels and HBV viral loads, the severity of liver disease assessed by Child-Pugh score (CP score) and degree of liver fibrosis as evaluated by transient elastography. In the current study, inadequate serum 25-hydroxyvitamin D_3 levels were found in 90% of HBV patients compared to 81.6% in controls and the mean level of serum 25-hydroxyvitamin D_3 was significantly lower among HBV cases compared to con-

trols. These results are comparable to the prevalence of serum 25-hydroxyvitamin D_3 in HBV patients in other studies which reported that serum 25-hydroxyvitamin D_3 deficiency is frequent in HBV patients and may reach above 90% [1, 7]. Another study discovered that 35% had insufficient serum 25-hydroxyvitamin D_3 levels and 58% had deficient serum 25-hydroxyvitamin D_3 levels. Serum 25-hydroxyvitamin D_3 insufficiency (less than 10 ng/ml) was shown to be quite common in people with HBV infection in the northern region of China (84.03%) [4].

The mechanism of 25-hydroxyvitamin D_3 insufficiency in HBV patients is considered multi-factorial. A possible explanation might be that cirrhotic liver function is compromised, resulting in insufficient 25-hydroxyvitamin D_3 production and activation [4, 7]. Deteriorated liver function causes mitochondrial CYP27A1 protein levels to decrease substantially. On the other hand, microsomal CYP2R1, the main enzyme for 25-hydroxyvitamin D_3 production under normal physiological settings and which may be less impacted by liver dysfunction, is upregulated. In the context of low serum 25-hydroxyvitamin D_3 levels, the latter phenomenon might be compensatory. As a result, the imbalance of these hydroxylases may play a crucial role in the development of 25-hydroxyvitamin D_3 insufficiency in chronic liver disease patients [2, 4]. Another possible explanation is that the liver plays a critical role in vitamin D activation. The hydroxylation of vitamin D occurs in the liver. Furthermore, the liver synthesizes 25-hydroxyvitamin D_3-binding protein (DBP), the main carrier protein of 25OHD in the circulation. As a result, impaired liver function in HBV patients might have been a factor contributing to their low serum 25hydroxyvitamin D_3 levels [2]. Finally, through its early synthesis in the skin, sunlight is a key predictor of vitamin D status. HBV patients had considerably lower physical activity levels than the general population, which might contribute to reduced sunshine exposure and hence lower serum 25-hydroxyvitamin D_3 levels [2, 9].

High frequency of insufficient 25-hydroxyvitamin D_3 status in both healthy populations and patients with HBV-related liver disorders is a common finding in Egypt. Inadequate direct sunlight exposure (due to conventional, conservative dress patterns, etc.) and dietary habits are two major causes, both of which might inhibit 25hydroxyvitamin D_3 photosynthesis in the body.

The relationship between serum 25-hydroxyvitamin D_3 levels and HBV viral load was investigated in our study. Serum 25-hydroxyvitamin D_3 levels were found to be significantly and negatively associated with viral load. These results are similar to results obtained by several other studies [2, 3, 7, 10, 11]. HBV has been demonstrated in certain studies to suppress the expression of vitamin D receptors in HBV-infected cells, preventing vitamin D from assisting the immune defence system and, as a result, reducing the efficacy of viral replication inhibition [3]. Other studies failed to confirm any association between serum 25-hydroxyvitamin D_3 level and viral load [1, 4, 12-14]. This disparity might be explained mostly by patients' selection criteria. The host immune response has been shown to have a substantial impact on the pathogenesis of HBV infection. 25-Hydroxyvitamin D_3 insufficiency, which is common in HBV infection, is thought to fail to inhibit HBV replication. It has been hypothesized that a relationship exists between 25-hydroxyvitamin D_3 deficiency and susceptibility to different infections and the development of various inflammatory disorders. In addition, clinical investigations have associated low serum 25-hydroxyvitamin D_3 levels with a poor sustained virological response to interferon-based treatment in chronic hepatitis B and C [7]. 25-Hydroxyvitamin D_3 has been demonstrated to have an essential role in regulating both innate and adaptive immunity [7, 15]. 25-Hydroxyvitamin D_3 receptors are expressed by a variety of immune cells, including macrophages, B cells, T cells, and antigen-presenting cells [16], and the greatest amounts of 25-hydroxyvitamin D_3 receptors are seen in CD8+ lymphocytes, which are the determinant immune cells for HBV clearance [2]. According to He *et al.* [17], 25-hydroxyvitamin D_3 has a clear impact on cellular immunity in HBV patients treated with interferon. All of these observations point to the fact that 25-hydroxyvitamin D_3 plays a key role in the immune response to HBV and even HBV replication and viral load.

Our results showed that 25-hydroxyvitamin D_3 deficiency was strongly linked to the degree of severity of liver disease as assessed by the Child-Pugh score. The actual cause of this association, however, remains unknown. In previous studies, it was reported that serum 25-hydroxyvitamin D_3 levels of less than 10 ng/ml can be a predictor of chronic liver disease severity [7, 18]. According to recent research, cirrhotic patients had considerably lower serum 25-hydroxyvitamin D_3 levels than healthy controls, and this was associated with a stepwise decline in serum 25-hydroxyvitamin D_3 level with more severe liver disease. They concluded that low serum 25-hydroxyvitamin D_3 levels in HBV patients might indicate liver dysfunction and can be correlated with the severity of liver disease [4]. Recent studies reported that in individuals with HBV infection, 25-hydroxyvitamin D_3 insufficiency directly correlates with disease progression, and in HBV patients

serum 25-hydroxyvitamin D_3 level was found to be adversely associated with Model for End-Stage Liver Disease score. Furthermore, a decreased serum 25-hydroxyvitamin D_3 level was found to be an independent predictor of long-term negative consequences [2, 7, 14]. In our study, because we included a wide range of Child-Pugh categories, the relationship between 25-hydroxyvitamin D_3 and the severity of liver disease could be properly assessed.

This study clarified the correlation between serum 25-hydroxyvitamin D_3 level and the degree of liver fibrosis as assessed by transient elastography and demonstrated that the level of serum 25-hydroxyvitamin D_3 was strongly associated with the degree of liver fibrosis. Another study on individuals with chronic liver disease discovered a link between serum 25-hydroxyvitamin D_3 levels and liver stiffness measured by transient elastography [1]. Furthermore, multivariate analysis revealed that serum 25-hydroxyvitamin D_3 level was substantially related to advanced hepatic fibrosis [5]. This is contradictory to results obtained from some other studies which reported that 25-hydroxyvitamin D_3 was not associated with the degree of liver fibrosis [12]. The overall influence of 25-hydroxyvitamin D_3 on the immunological signature in the setting of viral infections is an emerging field of research [19]. Abramovitch *et al*. [20] found that 25-hydroxyvitamin D_3 decreased the growth of primary hepatic stellate cells and significantly reduced collagen expression in their experimental model. In autoimmune hepatitis, severe 25-hydroxyvitamin D_3 insufficiency was found to be a predictive biomarker [21].

Our results further suggest that in subjects with no history of previous diabetes, HBV is associated with IR. HOMA-IR was significantly higher in patients with HBV compared to the control group. This finding is in line with a previous paper reporting that HBV was found to be linked to IR [6]. According to a previous animal study, the insulin signalling pathway is impaired in HBV [22]. These findings suggest that individuals with HBV should be closely monitored for the development of IR and diabetes mellitus. Although in our study the degree of IR was found to be correlated with viral load, the degree of liver disease severity and fibrosis stage, longitudinal studies on the effects of IR on HBV natural history are needed.

The strength of our study is based on the fact that the question of the causal relationship between 25-hydroxyvitamin D_3 and HBV replication was addressed by our case-control study, whereas most studies on the relation between HBV-related illness and 25-hydroxyvitamin D_3 deficiency have been cross-sectional, so the causative relationship could not be proven. Since 25-hydroxyvitamin D_3 deficiency is prevalent in Egypt, even among the normal population, we included 60 normal control subjects for standardization. However, there are a few limitations that need to be addressed. First, individuals infected with genotype B were shown to have a greater frequency of 25-hydroxyvitamin D_3 deficiency than those infected with genotype C [1]. The current study, however, lacked HBV genotype data. More research is needed to determine whether different HBV genotypes have distinct relationships with serum 25-hydroxyvitamin D_3 levels. Second, a liver biopsy was not used to determine the extent of hepatic fibrosis. Instead, we depended on transient elastography to evaluate the degree of fibrosis since it is a more convenient modality for identification of the degree of liver fibrosis in daily clinical practice. Third, seasonal variations, sunlight exposure, geographical residence, and nutrition all have an impact on 25-hydroxyvitamin D_3 levels. However, there was no information available on these variables in our research. Furthermore, a variety of additional factors that influence IR, including skeletal muscle condition and physical activity, were not explored in the current study. To validate our findings, further research that overcomes these limitations is required.

Conclusions

This case-control study showed that HBV patients had significantly lower serum 25-hydroxyvitamin D_3 levels compared to healthy individuals, and that serum 25-hydroxyvitamin D_3 levels were inversely related to HBV viral loads, the severity of liver disease, and degree of liver fibrosis. Further longitudinal prospective studies are needed to evaluate the effects of 25-hydroxyvitamin D_3 administration on the course of HBV infection. HBV infection is associated with IR as identified by HOMA-IR. IR is found to be associated with the HBV viral loads, the severity of liver disease, and the degree of liver fibrosis.

Disclosure

The authors declare no conflict of interest.

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