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Vitamin D levels in patients with chronic hepatitis B virus infection and naturally immunized individuals

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Abstract

Vitamin D deficiency is associated with several adverse health outcomes, and vitamin D appears to have systemic antimicrobial effects that may be crucial in a variety of both acute and chronic illnesses. In the present study, 25-hydroxyvitamin D (25-OHD) levels were compared among patients with chronic hepatitis B virus infection, naturally immunized individuals and control individuals. Thirty-five patients with chronic hepatitis B virus infection (group I), 30 naturally immunized individuals (group II) and 30 healthy adults were included in the present study. Markers of hepatitis were measured using commercially available kits based on chemiluminescence assays. Routine biochemical parameters, hepatitis B virus serology, hepatitis B virus DNA, 25-OHD and parathyroid hormone levels were measured. Baseline characteristics of the study groups were comparable. Patients in group I had a lower 25-OHD level compared with group II and the control group (7.65±4.19 ng/mL versus 12.1±7.13 ng/mL and 14.17±9.18 ng/mL, respectively; P<0.001). In addition, patients in group I had a higher parathyroid hormone level compared with group II and the control group (88.21±34.2 ng/mL versus 75.14±23.4 ng/mL and 74.16±20.15 ng/mL, respectively; P=0.001). 25-OHD levels were correlated with hepatitis B virus DNA levels. In patients infected with hepatitis B virus, diminished 25-OHD levels may be an indicator of the status of viral replication and portends a poor prognosis.

Keywords: Hepatitis B, immune system, vitamin D

Introduction

Hepatitis B virus (HBV) infection is a major public health problem worldwide. Hepatitis B is an infectious disease, affecting an estimated 350 million chronically infected patients [1,2].

HBV is a 42 nm DNA virus belonging to the family Hepadnaviridae. The virus has a partially double-stranded DNA genome and contains a core antigen (HBcAg) surrounded by a shell containing surface antigen (HBsAg). The immune response to HBsAg provides immunity against HBV. Antibodies to HBcAg (anti-HBc) indicate infection; immunoglobulin (Ig) M anti-HBc indicates recent infection and usually disappears within six months, while IgG anti-HBc persists for life and indicates past infection. Antibodies to HBsAg (anti-HBs) appear after clearance of HBsAg or after immunization. The presence of HBsAg for longer than six months is defined as chronic HBV infection [3].

The clinical course of hepatitis B is determined by the interaction between viral replication and the host immune response. HBV infection is generally asymptomatic; however, HBV is the most common and important cause of cirrhosis and hepatocellular carcinoma worldwide [2,4]. Vitamin D deficiency is associated with several adverse health outcomes. A plethora of health benefits associated with vitamin D supplementation, including a boost in longevity, are evident. Vitamin D has an emerging role in regulating inflammation as well as an important role in immunomodulation. Vitamin D may also improve survival in acute illness by boosting innate immunity, and appears to exhibit systemic antimicrobial effects that may be crucial in a variety of both acute and chronic illness [5-8]. Given this information, we hypothesized that vitamin D deficiency is related to HBV infection status and is a prognostic marker.

To our knowledge, the present study was the first to assess the relationship between vitamin D deficiency in patients with HBV infection and immune response. The aim of the present study was to define the pattern of vitamin D levels in patients with chronic HBV infection compared with naturally immunized individuals.

Materials and methods

Patient selection

Thirty-five patients who had been followed in the outpatient clinic of the infection diseases department due to chronic hepatitis B (HBsAg positive, anti-HBs negative for at least six months), who had normal liver enzyme levels and had not received antiviral treatment (group I; mean (± SD) age 32.5±9.8 years; 22 men), and 30 naturally immunized individuals (HBsAg negative, anti-HBs and anti-HBc-IgG positive) (group II; mean age 31.1±5.5 years; 18 men) were included in the present study. Thirty age-matched healthy adult subjects were also included as a control group (mean age 32.4±8.4 years; 17 men).
Because the level of 25-hydroxyvitamin D (25-OHD) fluctuates according to seasonal changes (effects of sunlight), the study was initiated in the winter season and continued to the end of March.

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Table 1. Comparison of clinical and biochemical features of HBV patients and controls.

	Group I (n=35)	Group II (n=30)	Control (n=30)	P value
age (years) sex	32.5 ± 9.8	31.1 ± 5.5	32.4 ± 8.4	NS
(males) (n,%)	22 (55%)	18 (60%)	17 (56.6%)	NS
body mass index (kg/m²)	22.96 ± 3.35	22.51 ± 2.85	23.49 ± 4.39	NS
creatinin(mg/dl)	0.89 ± 0.9	0.78 ± 0.8	0.75 ± 0.8	NS
hemoglobin(g/dl)	14.1 ± 1.4	13.1 ± 1.3	13.9 ± 1.3	NS
AST(mg/dl)	29.17 ± 3.18	26.7 ± 2.15	27.8 ± 3.4	NS
ALT (mg/dl)	31.25 ± 3.9	33.16 ± 2.3	31.9 ± 3.14	NS
TSH(mcIU/ml)	1.35 ± 1.1	1.22 ± 1.09	1.52 ± 1.45	NS
parathormone (pg/ml)	88.21 ± 34.2	74.16 ± 20.15	75.14 ± 23.4	0.001
25OHvitaminD (ng/ml)	7.65 ± 4.19	14.17 ± 9.18	12.1 ± 7.13	<0.001

Table 2. Comparison of vitamin D levels according to HBV DNA in chronic HBV group Controls.

HBVDNA(IU/ml)	n	vitamin D (mg/dl)
< 6 IU/ml	2	9.32± 4.26
6-1000 IU/ml	12	8.96 ± 4.15
1000-1000000 IU/ml	15	6.62 ± 3.21
>1000000 IU/ml	6	5.13 ± 2.7

Patients with chronic renal failure, chronic liver disease, cardiac failure (ejection fraction <50%), bone disorders, thyroide disorders, previous gastrectomy or having intestinal malabsorption and taking calcium, vitamin D or antidepressant drugs, hepatitis C, hepatitis D, HIV infection, and systemic bacterial or fungal infection, and other causes of liver disease, such as alcohol consumption and autoimmune hepatitis, were excluded from the present study.

Laboratory tests

Serum parathyroid measurements were performed using an electrochemiluminescence-based method on an E 170 Modular Analytic System (Roche, USA) device. 25-OHD levels were measured using a 25OH-Vitamin D3-Ria-CT Kit (Biosource Europe, Belgium). Reference ranges for 25-OHD were 10 ng/mL to 50 ng/mL for the winter season and 20 ng/mL to 120 ng/mL for the summer season [9]. Hepatitis markers were determined using commercially available kits based on chemiluminescence assays. HBV DNA was quantified using the PCR Cobas Taqman 48 system (Roche, USA).

Statistical analysis

Statistical analyses were performed using SPSS version 13 (IBM Corporation, USA) and Epi Info (Centers for Disease Control and Prevention, USA). Numerical variables are presented as median ± SD and categorical variables are presented as percentages. The normality of the data was tested using the Shapiro-Wilk test. Because the data were not normally distributed, the Mann-Whitney U test, a nonparametric statistical test, was used to compare the mean values among the groups. Categorical variables were compared using the $\chi 2$ test or Fisher's exact $\chi 2$ test. For all statistical studies, $P<0.05$ was considered to be statistically significant.

Results

Evaluating basic characteristics, there were no statistically significant differences among the three groups in terms of age, sex distribution, body mass index, smoking, or creatinine, aspartate aminotransferase, alanine aminotransferase and thyroid-stimulating hormone levels (**Table 1**).

With regard to the main biochemical parameters, patients in group I had lower vitamin D levels compared with group II and the control group (7.65±4.19 ng/mL versus 12.1±7.13 ng/mL and 14.17±9.18 ng/mL, respectively; P<0.001) and group I patients had higher parathyroid hormone levels compared with group II and the control group (88.21±34.2 pg/mL versus 75.14±23.4 pg/mL and 74.16±20.15 pg/mL, respectively; P<0.001) (**Table 1**).

In addition, when HBV-DNA levels were determined, vitamin D levels were correlated with HBV-DNA levels (**Table 2**).

Discussion

Recent studies have revealed functions of vitamin D in addition to those in bone metabolism. It has been found to be involved in autoimmune disorders such as inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis, psoriasis, diabetes, certain cancer types, hypertension, heart failure, atherosclerosis, peripheral artery disease and several infectious diseases [10].

Vitamin D directly leads to the expression of vitamin D receptor and CYP27B1 in vascular smooth muscle cells and in endothelial cells [11].

Recently, it has been recognized that vitamin D has other functions in addition to its role in bone metabolism. It has been demonstrated that vitamin D deficiency may play a role in the development of autoimmune diseases, inflammatory bowel disease, rheumatoid arthritis, psoriasis, multiple sclerosis, diabetes, certain cancer types, cardiac failure, stroke and infectious diseases such as tuberculosis and pneumonia, and that vitamin D supplementation is efficacious in these patients [12-16].

There is evidence that vitamin D may have a protective role in influenza and other viral diseases and may decrease the risk of developing AIDS in HIV-positive patients, hepatitis and other viral infections [17-20].

Sabetta *et al.,* [21] demonstrated that maintenance of a vitamin D serum concentration of 38 ng/mL or higher could significantly reduce the incidence of acute viral

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respiratory tract infections, including influenza, at least during the fall and winter in temperate zones.

In Indian children younger than five years of age, subclinical vitamin D deficiency was a significant risk factor for severe acute lower respiratory tract infections [22].

Chronicity of hepatitis B infection is also influenced by mutations in the vitamin D receptor gene, with polymorphisms being associated with higher viral load and increased disease progression and severity. Of note, the t allele is associated with enhanced Th1 cellular immunity and promotes more efficient clearance of several viral infections, including hepatitis B and dengue virus [23,24]. One study involving patients with hepatitis C virus demonstrated that vitamin D inhibits viral RNA replication, reportedly by inducing oxidative stress in a manner similar to the action of cyclosporine [25]. Petta *et al.,* [26] demonstrated that low serum 25-OHD levels were associated with risk of severe fibrosis and low sustained viral response to interferon treatment in patients chronically infected with genotype 1 hepatitis C virus. Another study also showed that vitamin D supplementation improves response to antiviral treatment for recurrent hepatitis C [27]. Vitamin D is linked not only to liver fibrosis but also to liver cirrhosis. A significant correlation exists between polymorphisms in the vitamin D receptor gene and the occurrence of hepatocellular carcinoma in patients with liver cirrhosis; this association is even more prominent in alcoholic patients [28,29].

In the present study, vitamin D levels were examined in patients with chronic HBV infection and naturally immunized individuals. Vitamin D levels were found to be lower in the chronic hepatitis B patients compared with naturally immunized individuals and control individuals ($P<0.001$).

When the three groups were compared in our study, 25-OHD levels of the patients with chronic hepatitis B were significantly lower than the other groups ($P<0.001$). In addition, we found a relationship between vitamin D levels and viral load (HBV-DNA). Our present data show that vitamin D deficiency may be related to increased viral replication in patients with HBV infection.

Kaleli *et al.,* [30] showed that neopterin levels, as a marker for immune activation, were higher in replicative HBV carriers.

Vitamin D is known to suppress proinflammatory cytokines and cause an increase in interleukin-10 levels [11]. Because of these effects, it is believed that vitamin D deficiency may be related to the development of increased viral replication. In our study, when the three groups were compared, levels of parathyroid hormone in the replicative HBV patients were significantly higher than those of the nonreplicative patients and controls ($P=0.001$).

As a result, our study revealed a relationship between vitamin D deficiency and viral replication in patients with chronic HBV infection. However, 25-OHD levels were found to be similar in the group with previous HBV infection (the naturally immunized group) and the control group. This suggests that vitamin D deficiency may increase viral replication and vitamin D supplementation may be useful in patients with chronic HBV infection.

The most important limitation of our study was the small number of patients. There is a need for large-scale research into this issue.

Abbreviations

HBV: hepatitis B virus
HCV: hepatitis C virus
OH: hydroxy
DNA: deoxyribonucleic acid
IgM: immunoglobulin M
IgG: immunoglobulin G
HBsAg: hepatitis B virus surface antigen
PTH: parathormone
AST: aspartate aminotransferase
ALT: alanine aminotransferase
TSH: Thyroid-stimulating hormone
VDR: vitamin D receptors
IFN: interferon
IL: interleukin

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Canan Demir participated in the acquisition and analysis and interpretation of data and drafting the manuscript. Mehmet Demir participated in the design of the study and revising the manuscript critically for important intellectual content. Both authors read and approved the final manuscript.

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References

1. Lavanchy D: **Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures**. *J Viral Hepat* 2004, **11**:97-107. | Article | PubMed
2. Kwon SY and Lee CH: **Epidemiology and prevention of hepatitis B virus infection**. *Korean J Hepatol* 2011, **17**:87-95. | Article | PubMed Abstract | PubMed Full Text
3. Fattovich G: **Natural history and prognosis of hepatitis B**. *Semin Liver Dis* 2003, **23**:47-58. | Article | PubMed
4. Ganem D and Prince AM: **Hepatitis B virus infection--natural history and clinical consequences**. *N Engl J Med* 2004, **350**:1118-29. | Article | PubMed
5. Holick MF: **Vitamin D: evolutionary, physiological and health perspectives**. *Curr Drug Targets* 2011, **12**:4-18. | Article | PubMed
6. Thomas DR: **Vitamins in aging, health, and longevity**. *Clin Interv Aging* 2006, **1**:81-91. | PubMed Abstract | PubMed Full Text
7. Fukuoka M, Ogino Y, Sato H, Ohta T, Komoriya K, Nishioka K and Katayama I: **RANTES expression in psoriatic skin, and regulation of RANTES and IL-8 production in cultured epidermal keratinocytes by active vitamin D3 (tacalcitol)**. *Br J Dermatol* 1998, **138**:63-70. | Article | PubMed
8. Kamen DL and Tangpricha V: **Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity**. *J Mol Med (Berl)* 2010, **88**:441-50. | Article | PubMed Abstract | PubMed Full Text

doi: 10.7243/2052-6954-1-2

9. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T and Dawson-Hughes B: **Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes**. *Am J Clin Nutr* 2006, **84**:18-28. | Article | PubMed
10. DeLuca HF: **Overview of general physiologic features and functions of vitamin D**. *Am J Clin Nutr* 2004, **80**:1689S-96S. | Article | PubMed
11. Cardus A, Parisi E, Gallego C, Aldea M, Fernandez E and Valdivielso JM: **1,25-Dihydroxyvitamin D3 stimulates vascular smooth muscle cell proliferation through a VEGF-mediated pathway**. *Kidney Int* 2006, **69**:1377-84. | Article | PubMed
12. Cannell JJ, Vieth R, Umhau JC, Holick MF, Grant WB, Madronich S, Garland CF and Giovannucci E: **Epidemic influenza and vitamin D**. *Epidemiol Infect* 2006, **134**:1129-40. | Article | PubMed Abstract | PubMed Full Text
13. Lorente F, Fontan G, Jara P, Casas C, Garcia-Rodriguez MC and Ojeda JA: **Defective neutrophil motility in hypovitaminosis D rickets**. *Acta Paediatr Scand* 1976, **65**:695-9. | Article | PubMed
14. Jeng L, Yamshchikov AV, Judd SE, Blumberg HM, Martin GS, Ziegler TR and Tangpricha V: **Alterations in vitamin D status and anti-microbial peptide levels in patients in the intensive care unit with sepsis**. *J Transl Med* 2009, **7**:28. | Article | PubMed Abstract | PubMed Full Text
15. Ginde AA, Mansbach JM and Camargo CA, Jr.: **Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey**. *Arch Intern Med* 2009, **169**:384-90. | Article | PubMed Abstract | PubMed Full Text
16. Schwalfenberg GK: **A review of the critical role of vitamin D in the functioning of the immune system and the clinical implications of vitamin D deficiency**. *Mol Nutr Food Res* 2011, **55**:96-108. | Article | PubMed
17. Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y and Ida H: **Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren**. *Am J Clin Nutr* 2010, **91**:1255-60. | Article | PubMed
18. de la Torre MS, Torres C, Nieto G, Vergara S, Carrero AJ, Macias J, Pineda JA, Caruz A and Fibla J: **Vitamin D receptor gene haplotypes and susceptibility to HIV-1 infection in injection drug users**. *J Infect Dis* 2008, **197**:405-10. | Article | PubMed
19. Lange CM, Bojunga J, Ramos-Lopez E, von Wagner M, Hassler A, Vermehren J, Herrmann E, Badenhoop K, Zeuzem S and Sarrazin C: **Vitamin D deficiency and a CYP27B1-1260 promoter polymorphism are associated with chronic hepatitis C and poor response to interferon-alfa based therapy**. *J Hepatol* 2011, **54**:887-93. | Article | PubMed
20. Chan MC, Cheung CY, Chui WH, Tsao SW, Nicholls JM, Chan YO, Chan RW, Long HT, Poon LL, Guan Y and Peiris JS: **Proinflammatory cytokine responses induced by influenza A (H5N1) viruses in primary human alveolar and bronchial epithelial cells**. *Respir Res* 2005, **6**:135. | Article | PubMed Abstract | PubMed Full Text
21. Sabetta JR, DePetrillo P, Cipriani RJ, Smardin J, Burns LA and Landry ML: **Serum 25-hydroxyvitamin d and the incidence of acute viral respiratory tract infections in healthy adults**. *PLoS One* 2010, **5**:e11088. | Article | PubMed Abstract | PubMed Full Text
22. Wayse V, Yousafzai A, Mogale K and Filteau S: **Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 y**. *Eur J Clin Nutr* 2004, **58**:563-7. | Article | PubMed
23. Bellamy R, Ruwende C, Corrah T, McAdam KP, Thursz M, Whittle HC and Hill AV: **Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the vitamin D receptor gene**. *J Infect Dis* 1999, **179**:721-4. | Article | PubMed
24. Loke H, Bethell D, Phuong CX, Day N, White N, Farrar J and Hill A: **Susceptibility to dengue hemorrhagic fever in vietnam: evidence of an association with variation in the vitamin d receptor and Fc gamma receptor IIa genes**. *Am J Trop Med Hyg* 2002, **67**:102-6. | Article | PubMed
25. Yano M, Ikeda M, Abe K, Dansako H, Ohkoshi S, Aoyagi Y and Kato N: **Comprehensive analysis of the effects of ordinary nutrients on hepatitis C virus RNA replication in cell culture**. *Antimicrob Agents Chemother* 2007, **51**:2016-27. | Article | PubMed Abstract | PubMed Full Text
26. Petta S, Camma C, Scazzone C, Tripodo C, Di Marco V, Bono A, Cabibi D, Licata G, Porcasi R, Marchesini G and Craxi A: **Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C**. *Hepatology* 2010, **51**:1158-67. | Article | PubMed
27. Bitetto D, Fabris C, Fornasiere E, Pipan C, Fumolo E, Cussigh A, Bignulin S, Cmet S, Fontanini E, Falleti E, Martinella R, Pirisi M and Toniutto P: **Vitamin D supplementation improves response to antiviral treatment for recurrent hepatitis C**. *Transpl Int* 2011, **24**:43-50. | Article | PubMed
28. Falleti E, Bitetto D, Fabris C, Cussigh A, Fontanini E, Fornasiere E, Fumolo E, Bignulin S, Cmet S, Minisini R, Pirisi M and Toniutto P: **Vitamin D receptor gene polymorphisms and hepatocellular carcinoma in alcoholic cirrhosis**. *World J Gastroenterol* 2010, **16**:3016-24. | Article | PubMed Abstract | PubMed Full Text
29. Youssef DA, Miller CW, El-Abbassi AM, Cutchins DC, Cutchins C, Grant WB and Peiris AN: **Antimicrobial implications of vitamin D**. *Dermatoendocrinol* 2011, **3**:220-9. | Article | PubMed Abstract | PubMed Full Text
30. Kaleli I, Demir M, Cevahir N, Yilmaz M and Demir S: **Serum neopterin levels in patients with replicative and nonreplicative HBV carriers**. *BMC Infect Dis* 2006, **6**:157. | Article | PubMed Abstract | PubMed Full Text