Journal of Medical Sciences

ISSN 1682-4474

science alert

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Research Paper

J. Med. Sci., 14 (4): 192-200
15th May, 2014
DOI: 10.3923/jms.2014.192.200

25-Hydroxyvitamin D_3 Level in Patients with Chronic Viral Hepatitis B

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Vitamin D is an important immune modulator that has an emerging role in inflammatory and metabolic liver diseases. An association has been established between low levels of vitamin D and several adverse health outcomes including upper respiratory and enteric infections, viral hepatitis and HIV infections. It exerts protective effects during infections by up-regulating the expression of cathelicidin and β-defensin 2 in phagocytes and epithelial cells. Thus, vitamin D appears to have systemic antimicrobial effects that may be crucial in a variety of both acute and chronic illnesses. In the current study, 25-hydroxyvitamin D_3 (25-OHD_3) levels were compared among 75 patients with chronic hepatitis B virus infection (Group I), sixty naturally immunized individuals (Group II) and another sixty age and sex-matched healthy controls. Routine biochemical parameters like hepatitis markers, hepatitis B virus serology, hepatitis B virus DNA, 25-OHD_3 and Parathormone levels were measured. Patients in group I had a significantly lower 25-OHD level compared with group II and controls (13.9±4.93 vs. 22.1±6.14 and 23.15±8.28 ng mL^{-1}, respectively $p<0.001$). In contrast, patients in group I had a higher parathyroid hormone level compared with group II and control group (103.14±24.5 vs. 75.14±23.4 and 74.1±20.15 pg mL^{-1}, respectively $p<0.001$). Also, 25-OHD levels were inversely correlated with hepatitis B virus DNA levels. The observed diminished 25-OHD levels in patients infected with hepatitis B virus may be an indicator of the viral replication status and portends a poor prognosis.

Key words: Hepatitis B, vitamin D

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

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INTRODUCTION

Hepatitis B Virus (HBV), a 42 nm DNA virus belonging to the family Hepadnaviridae, 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 (Fattovich, 2003).

Hepatitis B Virus (HBV) infection has been a major global cause of morbidity and mortality. The HBV infection remains one of the most significant infectious diseases worldwide. According to the World Health Organization, 2 billion people worldwide have been infected with HBV and roughly 600,000 die each year. Without antiviral therapy, the virus can attack the liver and chronic infections progress to liver disease and cirrhosis (Lavanchy, 2004). The interaction between viral replication and the host immune response determined the clinical course of hepatitis B. Hepatitis B shows variable clinical manifestations ranging from asymptomatic HBV carriers to fulminant liver failure and it becomes chronic, often progresses to chronic hepatitis, cirrhosis and hepatocellular carcinoma. Generally, HBV infection is asymptomatic. However, it constitute the most common and important cause of cirrhosis and hepatocellular carcinoma worldwide (Kwon and Lee, 2011).

Recently, as the prevalence of HBV infection is decreasing in young adult age group, the sexual contact has become the main transmission route. The association between low levels of vitamin D and several adverse health outcomes including upper respiratory and enteric infections has been established decade ago. Vitamin D_3 exerts protective effects during infections by up-regulating the expression of cathelicidin and β-defensin 2 in phagocytes and epithelial cells. Also, it is an important immune modulator that plays an emerging role in inflammatory and metabolic liver diseases, including Hepatitis C Virus infections (HCV) (Holick, 2011).

A plethora of health benefits associated with vitamin D supplementation, including a boost in longevity, are evident. Vitamin D has many emerging roles including anti-inflammatory, antimicrobial, immune-modulator and anti-apoptotic. Also, it improves 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 (Kamen and Tangpricha, 2010).

Vitamin D is very important in the maintenance of bone health and its deficiency is associated with many common and serious pathological conditions including cancer, autoimmune disease, cardiovascular disease, Insulin Resistance (IR), diabetes and leads to osteomalacia and contributes to fragility fractures (Petta *et al*., 2010). Deficiency has also been implicated in a wide variety of extra-skeletal conditions. Vitamin D can be easily assessed in patients by measuring serum 25-hydroxyvitamin D. Replacement of vitamin D needs to be tailored for each patient and depends on the severity of the deficiency. Toxicity is unlikely with vitamin D when it is administered as cholecalciferol as it has a wide safety window. The adequacy of replacement should be monitored and in cases of persistently low concentrations, malabsorptive conditions (especially celiac disease) should be excluded (Joshi *et al*., 2010).

Given these information, it is hypothesized that vitamin D deficiency may be related to HBV infection status and may be a prognostic marker. Vitamin D deficiency has been frequently reported in advanced liver disease, including hepatitis C virus (Ladero *et al*., 2013). However, the relationship between vitamin D metabolism and Chronic Hepatitis B (CHB) is less well characterized. To our knowledge, there is paucity in studies assessing 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 25-hydroxyvitamin D_3 levels in patients with chronic HBV infection compared with naturally immunized individuals and healthy controls.

MATERIALS AND METHODS

Materials: A total of 195 adult consecutive outpatient subjects, with age range of 18-70 years (Female/male: 99/96), with a confirmed diagnosis of chronic hepatitis B (n = 75) and naturally immunized individuals (n = 60) and another sixty age and sex-matched healthy control subjects were initially enrolled in this study. This study, in accordance with the World Medical Association (WMA) of Helsinki declaration (WMA, 2013), was approved by the Ethical Commission and Institutional Review Board of Mansura University Hospital in EGYPT. A written informed conscious consent was obtained from all patients before their participation.

Selection of patients: Chronic hepatitis B was diagnosed by HBs-Ag positive, anti-HBs negative for at least six months and positive HBV-DNA levels. Naturally immunized individuals were diagnosed as HBs-Ag negative, anti-HBs and anti-HBc-IgG positive.

'Inclusion criterion' was the diagnosis chronic hepatitis B. 'Exclusion criteria' were an age below 18 years and over 70 years, a history of cancer of any type within the last 5 years, a history of solid organ transplantation or previous bone marrow transplantation, antiviral treatment and local or systemic tumor-specific treatment within the last month. Patients with chronic renal failure, bone disorders, thyroid disorders, intestinal malabsorption, previous gastrectomy, taking vitamin D, calcium or antidepressant drugs, cardiac failure (ejection fraction <50%), hepatitis C, hepatitis D, HIV infection and systemic bacterial or fungal infection and other causes of liver disease, such as HCC, alcohol consumption, metabolic liver disease and autoimmune hepatitis, were excluded from the present study.

Initially, all patients completed a detailed questionnaire regarding diet and habits, submitted to thorough history taking with detailed physical examinations. The Model of End-stage Liver Disease (MELD score) (Kamath *et al*., 2001) and ChildBPugh score (Pugh *et al*., 1973), laboratory parameters and the results of ultrasound, CT scans and MRI imaging were assessed at the time of inclusion in the study. 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. All participants were assigned to the following groups:

- **Group I:** Comprised 75 CHB patients (age: 49.77±8.9 years; Females/Males: 39/36)
- **Group II:** Comprised 60 naturally immunized individuals (age: 48.1±7.6 years; Females/Males: 31/29)
- **Control group:** Comprised 60 healthy controls (age: 47.9±8.3 years; Females/Males: 29/31)

Biochemical profile: At the day of study inclusion, blood samples were obtained from each subject. Serum tubes were centrifuged at 1500 g for 10 min at 4°C, followed by a second centrifugation at 2000 g for 3 min at 4°C, aliquoted and stored at -80°C until assayed:

- 25OH-Vitamin D_3 (25-OHD) levels were measured using a 25OH-Vitamin D_3-direct ELISA Kit intended for the quantitative determination of the 25-OHD in serum and fresh plasma (Crystal Chem, INC. Catalog. No: 09002, assay range: 0-200 ng mL^{-1}, assay time <2 h, Precision CV: <10%, storage: 2-8°C) (Wielders and Wijnberg, 2009). Samples were measured in duplicates on a Tecan SLT Rainbow plate reader (Tecan, Männedorf, Switzerland)
- Parathormone (PTH) and Thyroid Stimulating Hormone (TSH) levels were measured using an electrochemiluminescence-based method on an E 170 Modular Analytic System (Roche, USA) device
- Assessment of HBV Status, based on anti-HBc and HBsAg test results, HBV status was defined as chronic (anti-HBc, HBsAg positive, anti-HBs negative for at least six months and positive HBV-DNA levels), immune (HbsAg negative, anti-HBs and anti HBc IgG positive) or HBV negative (anti-HBc negative)
- HBV-DNA levels were quantified using the PCR Cobas Taqman 48 system (Roche, USA). 'Hepatitis markers' were determined using commercially available kits based on chemiluminescence assays

Statistical analysis: Data were analyzed using SPSS software (Version 17.0). Quantitative data were expressed as (Mean±SD) while qualitative data and categorical variables were expressed as number and percentage. Continuous data are expressed as median (range) were evaluated by appropriate statistical tests, t-test (for paired data). Proportions were compared by means of Fisher's exact test. Correlations were evaluated using the Spearman rank correlation coefficient test. Kruskal-Wallis one way analysis of variance (ANOVA) compares more than two groups. Subgroups (percentages of patients) were compared by using the McNemar test. Categorical variables were compared using the χ^2 test or Fisher's exact χ^2 test. Variables that achieved statistical significance with the univariate analysis were included in multiple regression analysis with forward stepwise (likelihood ratio) to evaluate the independent factors associated with low 25OHD3 levels. Sensitivity, specificity and predictive values were calculated to study the overall predictability and accuracy of other techniques. For all statistical studies, $p<0.05$ was considered to be statistically significant.

RESULTS

The clinical and biochemical features of HBV patients and controls were shown in Table 1. There were no statistically significant differences among the three groups in terms of age, sex distribution, body mass index, creatinine, liver aminotransferase (ALT and AST) and thyroid-stimulating hormone levels. Serum 25 (OH)

Table 1: Comparison of demographic, clinical and biochemical features of HBV patients and controls

Parameters	Group I (CHB) (n = 75)	Group II (Naturally immunized) (n = 60)	Healthy control (n = 60)	p-value
Age (years)	49.77±8.9	48.1±7.6	47.9±8.3	ns
Sex (Females/Males)	39/36	31/29	29/31	ns
Body mass index (kg m^{-2})	22.3±2.55	23.21±2.26	22.94±4.45	ns
Creatinine (mg dL^{-1})	1.09±0.297	1.03±0.7	0.98±0.8	ns
Hemoglobin (g dL^{-1})	13.7± 0.94	13.4±1.5	14.3±1.2	ns
Platelets ($\times 10^3$ cm^{-2})	233.5±19.2	236.7±22.4	237.5±17.2	ns
S. Albumin (g dL^{-1})	4.09±0.48	5.01±0.32	4.3±0.34	ns
Aspartate aminotransferase, AST (mg dL^{-1})	39.1±6.53	37.7±3.19	35.8±5.2	ns
Alanine aminotransferase, ALT (mg dL^{-1})	30.07±5.3	33.16±1.8	32.7±4.2	ns
Thyroid stimulating hormone, (mcIU mL^{-1})	1.46±0.3	1.6±0.09	1.26±1.01	ns
Parathormone (pg mL^{-1})	103.14±24.5	75.14±23.4	74.16±20.15	0.001
25-Hydroxy-vitamin D_3 (ng mL^{-1})	13.9±4.93	22.1±6.14	23.15±8.28	<0.001
HBV-DNA levels (IU $mL^{-1}\times 10^3$)	882.04±572.1	-	-	-

Table 2: Demographic, clinical and biochemical characteristics in patients with chronic Hepatitis B according to 25OH vitamin D_3 levels

	25OH vitamin D_3			ANOVA	
Parameters	Normal: >20 ng mL^{-1} (n = 9)	Insufficiency: 10-20 ng mL^{-1} (n = 44)	Deficiency:<10 ng mL^{-1} (n = 22)	F	p
Number (%)	9 (12%)	44 (58.7%)	22 (29.3%)		
25-Hydroxy-Vitamin D_3 (ng mL^{-1})	22.3±2.13	13.98±2.92	8.38±4.87	36.05	<0.0010
Age (years)	50.00±8.6	48.41±8.26	52.41±9.9	1.51	0.2260
Sex (Mean±SD, Females/Males)	0.555±0.52; 4/5	0.52±0.51; 21/23	0.36±0.49; 14/8	0.84	0.4340
Body mass index (kg m^{-2})	22.22±3.27	22.36±2.47	22.3±2.51	0.01	0.9880
Creatinine (mg dL^{-1})	1.188±0.34	1.03±0.27	1.18±0.31	2.36	0.1210
Hemoglobin (g dL^{-1})	14.15±0.94	13.7±0.99	13.6±0.79	1.24	0.2950
Platelets ($\times 10^3$ cm^{-2})	233.3±29.47	234.3±17.3	235.7±18.66	0.06	0.9440
S. Albumin (g dL^{-1})	4.68±0.358	5.09±0.49	4.98±0.475	2.94	0.0600
Aspartate aminotransferase (mg dL^{-1})	41.2±5.995	38.8±7.29	38.8±5.06	0.53	0.5910
Alanine aminotransferase (mg dL^{-1})	33.2±5.517	29.2±5.13	30.5±5.06	2.38	0.1100
Thyroid stimulating hormone (µU mL^{-1})	1.54±0.353	1.41±0.32	1.52±0.274	1.23	0.2980
Parathormone (pg mL^{-1})	81.3±27.76	99.04±20.4	120.3±20.48	12.58	<0.0010
HBV-DNA levels (IU $mL^{-1}\times 10^3$)	0.475±0.312	683.3±579.9	989.6±472.1	12.26	<0.0001

Data expressed as Mean±SD

vitamin D_3 concentrations <10 ng mL^{-1} were defined as severe vitamin D deficiency; levels from 10-20 ng mL^{-1} were considered as insufficiency and serum levels >20 ng mL^{-1} were considered normal. A significantly lower 25-Hydroxy-Vitamin D_3 levels were observed in patients of group I compared with group II and control group (13.9±4.93 vs. 22.1±6.14 and 23.15±8.28 ng mL^{-1}, respectively $p<0.001$). Also, patients in group I had statistically significantly higher parathyroid hormone levels compared with group II and the control group (103.14±24.5 vs. 75.14±23.4 and 74.16±20.15 pg mL^{-1}, respectively $p<0.001$).

The comparison of demographic, clinical and biochemical characteristics in patients with chronic hepatitis B according to 25OH Vitamin D_3 levels were shown in Table 2. No significant differences were observed in any of these parameters in relation to 25OH Vitamin D_3 levels except for HBV-DNA level and Parathormone. In contrast to Parathormone, HBV-DNA level was found to have a significant inverse association with 25OH Vitamin D_3 levels ($p<0.001$).

The comparison of demographic, clinical and biochemical characteristics in patients with chronic hepatitis B according to HBV-DNA levels were shown in Table 3. No significant differences were observed in any of these parameters in relation to HBV-DNA levels except for 25-OH vitamin D_3 and Parathormone. In contrast to Parathormone, 25OH vitamin D_3 level was found to have a significant inverse association with HBV-DNA levels ($p<0.001$). The serum 25OH vitamin D_3 concentration was 16.9"4.1 ng mL^{-1} for patients with HBV DNA<1000 IU mL^{-1}, compared with 8.5±1.94 ng mL^{-1} for those with ≥2000 IU mL^{-1}, a significant difference.

Spearman correlations of 25OH vitamin D_3 levels, HBV-DNA levels and different clinical and biochemical features in chronic HBV patients were shown in Table 4. 25-Hydroxy-Vitamin D levels were significantly and negatively correlated with HBV-DNA levels, Parathormone and Serum albumin but positively correlated with aminotransferase (ALT and AST). In both univariate and multivariate analyses, HBV-DNA was the major determinant factor of low 25OH vitamin D_3 concentration (OR (95% CI): 3.13 (1.92-5.12), p = 0.005 and 3.13 (1.92-5.12), p = 0.0007, respectively) (Table 5).

Table 3: Demographic, clinical and biochemical characteristics in patients with chronic hepatitis B according to HBV-DNA levels

	HBV-DNA levels (IU mL^{-1})				
Parameters	Negative: <6 IU mL^{-1}	Mild: 6-10^3 IU mL^{-1}	Moderate: 1000-10^6 IU mL^{-1}	Severe: >10^6 IU mL^{-1}	ANOVA
Number (%)	4 (5.3%)	10 (13.3%)	21 (28%)	40(53.4%)	
Age (years)	55.5±4.04	48.2±7.89	48.95±8.5	50.03±9.6	0.541
Sex (Females/Males)	3/1	5/5	10/11	21/19	0.804
Body mass index (kg m^{-2})	25.0±1.15	21.6±2.7	23.47±2.35	21.65±2.4	0.105
Creatinine (mg dL^{-1})	1.30±0.23	1.15±0.32	1.09±0.32	1.06±0.29	0.504
Hemoglobin (g dL^{-1})	13.3±0.06	14.1±0.99	13.7±0.84	13.7±1.01	0.640
Platelets (×10^3 cm^{-2})	245±9.12	236±32.8	235.7±19.7	232.6±15.1	0.387
S. Albumin (g dL^{-1})	4.53±0.03	4.6±0.38	4.76±0.45	5.29±0.36	0.145
Aspartate aminotransferase (mg dL^{-1})	42.5±6.13	42±6.49	42.6±7.9	36.2±4.19	0.242
Alanine aminotransferase (mg dL^{-1})	36.5±0.58	34.1±5.7	32.3±5.18	27.2±3.39	0.288
Thyroid stimulating hormone (µU mL^{-1})	1.3±0.23	1.6±0.38	1.48±0.27	1.43±0.31	0.076
Parathormone (pg mL^{-1})	81±22.2	98.7±33.9	112.8±20.6	101.4±22.5	0.011
25-Hydroxy-Vitamin D$_3$ (ng mL^{-1})	23.8±1.7	16.9±4.1	11.6±3.32	8.5±1.94	0.001

Data expressed as Mean±SD

Table 4: Spearman's rho correlations (r) between different parameters in patients with chronic hepatitis B

	TSH		PTH		25OH vitamin D$_3$		HBV-DNA levels	
	r	p	r	p	r	p	r	p
Age (years)	0.046	0.694	0.023	0.846	0.029	0.807	-0.022	0.852
Sex (Males); n (%)	0.012	0.916	-0.008	0.945	0.057	0.625	0.129	0.805
Body mass index (kg m^{-2})	-0.110	0.077	-0.103	0.379	-0.216	0.252	0.213	0.058
Creatinine (mg dL^{-1})	-0.132	0.258	0.011	0.927	0.010	0.929	-0.197	0.093
Hemoglobin (g dL^{-1})	-0.025	0.832	-0.149	0.204	0.117	0.319	0.100	0.398
Platelets (×103 cm^{-2})	-0.200	0.085	0.055	0.641	0.015	0.900	-0.146	0.213
S. Albumin (g dL^{-1})	0.003	0.976	-0.01	0.931	0.372*	0.001	-0.556*	0.008
Aspartate aminotransferase (mg dL^{-1})	0.037	0.752	0.033	0.776	-0.333*	0.004	0.465*	0.005
Alanine aminotransferase (mg dL^{-1})	0.038	0.746	0.029	0.806	-0.336*	0.003	0.532*	0.004
Thyroid stimulating TSH (µIU mL^{-1})	1.000	.	0.245*	0.034	-0.112	0.342	-0.066	0.576
Parathormone PTH (pg mL^{-1})	0.245*	0.034	1.000	.	-0.324*	0.005	-0.004	0.975
HBV-DNA levels (IU mL^{-1}×103)	-0.066	0.576	-0.004	0.975	-0.647*	0.001	1.000	.

*Correlation is significant if p-value≤ 0.05 level (2-tailed)

Table 5: Logistic multivariate regression analysis of determinant factors associated with 25OH vitamin D$_3$ in CHB patients

	25OH vitamin D$_3$				
Parameters	Normal: >20 ng mL^{-1} (n = 9)	Insufficiency: 10-20 ng mL^{-1} (n = 44)	Deficiency: <10 ng mL^{-1} (n = 22)	OR (95% CI)	p-value
Age (years)	50.00±8.6	48.41±8.26	52.41±9.9	2.34 (1.33-4.19)	0.4120
Sex (Mean±SD; Females/Males)	0.555±0.52; 4/5	0.52±0.51; 21/23	0.36±0.49; 14/8	1.64 (1.05-2.55)	0.2510
Body mass index (kg m^{-2})	22.22±3.27	22.36±2.47	22.3±2.51	1.26 (0.72-1.88)	0.7580
Creatinine (mg dL^{-1})	1.188±0.34	1.03±0.27	1.18±0.31	1.78 (0.88-1.79)	0.2230
Hemoglobin (g dL^{-1})	14.15±0.94	13.7±0.99	13.6±0.79	1.68 (1.12-2.12)	0.2870
Platelets (×10^3 cm^{-2})	233.3±29.47	234.3±17.3	235.7±18.66	2.34 (1.33-5.19)	0.7980
S. Albumin (g dL^{-1})	4.68±0.358	5.09±0.49	4.98±0.475	1.64 (1.05-2.55)	0.1060
Aspartate aminotransferase (mg dL^{-1})	41.2±5.995	38.8±7.29	38.8±5.06	1.26 (0.72-1.88)	0.4350
Alanine aminotransferase (mg dL^{-1})	33.2±5.517	29.2±5.13	30.5±5.06	1.78 (0.88-1.79)	0.1120
Thyroid stimulating hormone (µIU mL^{-1})	1.54±0.353	1.41±0.32	1.52±0.274	1.68 (1.12-2.12)	0.2540
Parathormone (pg mL^{-1})	81.3±27.76	99.04±20.4	120.3±20.48	2.4 (1.86-3.290)	0.0140
HBV-DNA levels (IU mL^{-1}×10^3)	0.475±0.312	683.3±579.9	989.6±472.1	3.13 (1.92-5.12)	0.0007

OR (95% CI): Odd ratio (95% confidence interval)

DISCUSSION

Vitamin D is characterized as a regulator of homeostasis of bone and mineral metabolism, but it can also provide non-skeletal actions and is linked to many major human diseases because vitamin D receptors have been found in various tissues including the brain, prostate, breast, colon, pancreas and immune cells. Bone metabolism, modulation of the immune response and regulation of cell proliferation and differentiation are all biological functions of vitamin D. Vitamin D may play an important role in modifying the risk of cardio-metabolic outcomes, including Diabetes Mellitus (DM), insulin resistance, hypertension and cardiovascular disease (Schwalfenberg, 2011).

Basic science and epidemiological studies indicate that vitamin D has important roles in modulation, development and function of the immune system. In fact,

inadequate vitamin D and other nutrients during the development of the immune system may play a critical role in the development of autoimmune diseases. Evidences from animal models and prospective studies of rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus and type 1 DM suggest that vitamin D has an important role as a modifiable environmental factor in autoimmune diseases (Mathieu and Adorini, 2002).

An association has been established between low levels of vitamin D and several adverse health outcomes including upper respiratory and enteric infections, viral hepatitis and HIV infections. Several vitamin-D-related gene polymorphisms and vitamin-D-related metabolic and immune pathways could drive its crucial non skeletal actions. It exerts protective effects during infections by up-regulating the expression of cathelicidin and β-defensin 2 in phagocytes and epithelial cells. Thus, Vitamin D appears to have systemic antimicrobial effects that may be crucial in a variety of both acute and chronic illnesses. Supplementations of vitamin D may provide suitable management and act to ameliorate some human disorders (Falleti *et al*., 2010).

Recently, it has been illustrated that vitamin D has additional functions rather than its central role in bone metabolism. It has been demonstrated that vitamin D may be involved in immune-modulation and that its 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, hypertension, atherosclerosis, peripheral artery disease, stroke and several bacterial or viral infectious diseases such as tuberculosis, pneumonia and hepatitis (HCV, HBV) (DeLuca, 2004; Cannell *et al*., 2006; Jeng *et al*., 2009; Schwalfenberg, 2011). Of interest, vitamin D supplementation is proved to be efficacious in these patients.

It has been evidenced that vitamin D may have a protective role in influenza and other viral diseases and may decrease the risk of hepatitis and developing AIDS in HIV-positive patients (Urashima *et al*., 2010).

Wayse *et al*. (2004) demonstrated that subclinical vitamin D deficiency was a significant risk factor for severe acute lower respiratory tract infections in Indian children younger than five years of age. Vitamin D serum concentration maintained at 38 ng mL^{-1} or more has been demonstrated to significantly reduce the incidence of acute viral respiratory tract infections, such as influenza during the fall and winter in temperate zones (Sabetta *et al*., 2010).

There has been a lot of evidence during the last decade that vitamin D has numerous additional functions such as anti-proliferative, pro-apoptotic, differentiating, anti-angiogenic and anti-invasive roles in cancer. Previous studies have examined the impact and influence of various host genetic factors, HLA class I and II, cytokines (tumor necrosis factor-α, interleukin-10), mannose-binding lectin, vitamin D receptor and various genetic polymorphisms on susceptibility to HBV infection and risk of chronic infection, cirrhosis and hepatocellular carcinoma, with conflicting results (Suneetha *et al*., 2006; Borresen *et al*., 2011).

25 (OH) D_3 modulates and restrains innate immune responses and limits activation of monocytes and thereby reduces the production of pro-inflammatory cytokines including Tumour Necrosis Factor α (TNFα) (Han *et al*., 2013). The Vitamin D Receptor (VDR) mediates the immune-regulatory effects of 1, 25-dihydroxyvitamin D_3 (1, 25D3) including monocytes activation, stimulation of cellular immune responses and suppresson of immunoglobulin production and lymphocyte proliferation (Ehrchen *et al*., 2007; De La Torre *et al*., 2008). The expression of VDR in hepatocytes suggests its role in hepatocellular injury. Chronicity of hepatitis B infection is influenced by Vitamin D Receptor gene (VDR) mutations, with polymorphisms. Higher viral load and increased disease progression and severity were associated with mutations in VDR gene (Bellamy *et al*., 1999).

Of note, the tt genotype of a Single Nucleotide Polymorphism (SNP) at position 352 of the VDR gene has been associated with enhanced Th1 cellular immunity and promotes more efficient clearance of several viral infections, including hepatitis B and dengue virus and resistance to pulmonary tuberculosis (Loke *et al*., 2002; Nevado *et al*., 2007).

Genetic polymorphisms in VDR are significantly associated with the occurrence of HCC in patients with liver cirrhosis. This relationship is more prominent in and specific for patients with an alcoholic etiology (Falleti *et al*., 2010). Finkelmeier *et al*. (2014) concluded that 25OH vitamin D_3 deficiency is associated with advanced stages of hepatocellular carcinoma high mortality risk independently from the MELD score and high alpha-Fetoprotein levels and that it is a prognostic indicator for a poor outcome. They observed a negative correlation between 25 (OH) D_3 and CRP, as well as soluble CD163 levels, indicating that an enhanced inflammatory environment is associated with lower 25 (OH) D_3 concentrations.

Vitamin D has an emerging role in inflammatory and metabolic liver diseases. Vitamin D is linked not only to liver fibrosis but also to liver cirrhosis. Some studies have found low serum 25 OH vitamin D_3 levels in patients with chronic hepatitis and cirrhosis of different origins. Low

25 OH vitamin D_3 levels have been related to poor liver function because of the association between vitamin D status and hepatic function indexes or the stage of cirrhosis. The incidence of 25OH vitamin D_3 deficiency was demonstrated to increase in patients with chronic liver disease and corresponding to the severity of liver dysfunction (Arteh *et al*., 2010). Furthermore, lower 25 (OH) D_3 levels were found in patients with chronic viral hepatitis including hepatitis B and C and correlate with higher rates of replication of the hepatitis B virus (Farnik *et al*., 2013) and its administration may even inhibit in vitro HCV viral replication (Petta *et al*., 2010). In addition, vitamin D can be considered as a predictor of treatment outcome for interferon-based anti-viral therapy (Lange *et al*., 2011). In patients with hepatitis C virus, vitamin D was found to inhibit viral RNA replication by inducing the oxidative stress in a manner similar to the action of cyclosporine (Yano *et al*., 2007). Low serum levels of 25-OH vitamin D_3 were linked to risk of severe fibrosis and low sustained viral response to interferon-based therapy in patients chronically infected with genotype 1 hepatitis C virus (Petta *et al*., 2010). Another study also demonstrated that vitamin D supplementation effectively improves response to antiviral treatment for recurrent hepatitis C (Bitetto *et al*., 2011).

Chronic liver diseases, including HBV, are accompanied by enhanced activation of the innate immune system and vitamin D levels inversely correlate with the expression of toll-like receptors in monocytes, indicating an inverse correlation between vitamin D levels and systemic inflammation (Kitson and Roberts, 2012). Recently, lower 25 OH vitamin D_3 serum levels were demonstrated in patients with biopsy-proven nonalcoholic fatty liver disease (NAFLD) identifying an independent association between the histological characteristics of NAFLD and low 25(OH)D levels (Targher *et al*., 2007; Fisher and Fisher, 2007).

In keeping with these studies, several reports describe reduced bone mineral density in patients with chronic liver disease and cirrhosis (Monegal *et al*., 1997). Also, experimental studies also suggested a potential role of vitamin D in interfering with inflammatory response and fibrogenesis through interaction with its nuclear receptor (VDR) (Chiu *et al*., 2004).

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

Comparing the three groups in this work, patients with chronic hepatitis B had significantly lower levels of 25-OH vitamin D_3 than the other groups ($p<0.001$). Moreover, we observed a significant inverse relationship between vitamin D levels and viral load (HBV-DNA). The present data showed that vitamin D deficiency may be related to increased viral replication in patients with HBV infection.

Infection by Hepatitis B Virus (HBV) causes complicated immunological, biochemical and histological changes in host immune response against the virus which can be specific or non-specific. The nonspecific response occurs via cytokines or other substance. Kaleli *et al*. (2006) observed that neopterin levels, as a marker for the activation of cell mediated immunity, were higher in replicative HBV carriers. Vitamin D is known to suppress pro-inflammatory cytokines and increase interleukin-10 levels (Cardus *et al*., 2006). Thus, it could be suggested that vitamin D deficiency may be related to increased viral replication and viral load. In the present study, when the three groups were compared, levels of parathyroid hormone in the replicative HBV patients were significantly higher than those of the non-replicative patients and controls ($p = 0.001$). In contrast to vitamin D, parathyroid hormone levels were significantly positively correlated with viral load (HBV-DNA).

CONCLUSION

This study revealed a relationship between vitamin D deficiency and viral replication in patients with chronic HBV infection. However, 25-OH vitamin D_3 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 efficacious in patients with chronic HBV infection. We conclude that although it is possible that vitamin D deficiency is a just an epiphenomenon of advanced liver disease, the determination of 25 (OH) D_3 levels is a feasible additional tool in predicting increased viral replication in chronic HBV patients.

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