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The Relationship between Vitamin D Deficiency and The Severity of Liver Fibrosis in Chronic Viral Hepatitis B and C

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ABSTRACT

Background & Aims: Vitamin D is an important immune modulator and it potentially interferes with fibrogenesis. Previous data indicated an association between vitamin D deficiency, fibrotic stage and sustained virologic response rates in chronic viral hepatitis C (CHC). The aim of this study was to determine the relationship between vitamin D deficiency and the fibrotic stage in chronic hepatitis B (CHB) and CHC.

Methods: A cross-sectional study was conducted in CHB and CHC patients who underwent liver biopsy at Ramathibodi hospital between January 2011 and January 2012. For each patient, biochemical tests, 25-hydroxyvitamin D (25[OH]D) level and liver biopsy were performed. The histological staging of liver biopsy (by Metavir scoring system) were obtained to determine the association between vitamin D deficiency and the fibrotic stage in patients with CHB and CHC.

Results: Eighty-one patients were enrolled. Forty-eight and thirty-three patients had CHB and CHC, respectively. Mean vitamin D levels in CHB and CHC were 21.07 ± 6.47 ng/mL and 24.15 ± 9.15 ng/mL, which were strikingly lower than the level reported from the study in healthy Thai population (32.29 ± 0.4 ng/mL). Twenty-three (48%) and eleven (33%) of CHB and CHC patients were found to have vitamin D deficiency. The 25(OH)D deficiency tended to correlate with advance fibrosis stage (37.5%, 40%, and 52.1% in metavir fibrosis score F0-1, F2, F3-4 respectively, $p = 0.529$). From forward stepwise logistic regression, vitamin D deficiency tended to be associated with significant fibrosis (= F2 fibrosis stage) of CHB and CHC (odds ratio [OR], 1.11; 95% confidence interval [CI], 0.379-3.250; $p = 0.847$) and severe fibrosis (= F3-4 fibrosis stage) of chronic hepatitis B and C (OR, 1.81; 95% CI, 0.613-5.391; $p = 0.281$)

Conclusions: Vitamin D deficiency is highly prevalent in patients with CHB and CHC. The 25(OH)D serum levels deficiency have a trend for correlation with advance fibrosis stage.

Key words : Vitamin D deficiency, chronic viral hepatitis, fibrogenesis

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INTRODUCTION

Chronic hepatitis B (CHB) and Chronic hepatitis C (CHC) are one of the common causes of cirrhosis. Patients with cirrhosis of the liver are at high risk of a large variety of complications. Especially the development of portal hypertension, followed by gastroesophageal varicosis and ascites are potentially life threatening problems[1]. Several studies propose factors correlated with cirrhosis in chronic viral hepatitis. Vitamin D is one of them. Previous studies have reported results about serum concentrations of 25-hydroxyvitamin D (25[OH]D) and its relationship with the severity of liver disease. Arteh et al have found that severe vitamin D deficiency (<7 ng/mL) was more common among patients with cirrhosis compared with noncirrhotic patients[2]. Several reports show the correlation of an increased severity of cirrhosis and the low serum levels of 25(OH)D[3,4].

Recently, Petta et al. show that low 25(OH)D levels are independently associated with female sex and with severity of necroinflammatory activity in CHC. This study also offers the first evidence that low 25(OH)D serum levels, together with known risk factors for fibrosis severity, such as older age, low cholesterol levels, and high necroinflammatory activity are independently associated with the presence of severe fibrosis (Metavir scoring system F3-4) in CHC. Also it leads to the decline in response to treatment with peginterferon and ribavirin in patients with CHC genotype 1 after a period of six months which may reflect the relationship of vitamin D and fibrosis of the liver[3].

Vitamin D is known for its role in calcium metabolism and maintenance of healthy bones. Moreover it has important effects on the growth and differentiation of many cell types and immune system[5,6]. Different experimental models showed the role of immunoregulatory properties of vitamin D, via interaction with vitamin D receptor (VDR) protects against oxidative stress production can influence the migration, proliferation, and gene expression of fibroblasts and reduces the inflammatory and fibrogenic activity of liver stellate cells[7-9].

There are also studies showed that the relationship of vitamin D inhibits matrix metalloproteinases (MMP, a family of zinc-dependent endoproteinases that are involved in degradation of extracellular matrix components) and induces their inhibitors. Although metalloproteinase facilitated degradation of extra-cellular matrix proteins, it is essential in physiological processes such as remodeling and tissue repair. Inappropriate, prolonged, or excessive expression of these enzymes has deleterious consequences. Subsequently, vitamin D deficiency has been associated with the increase in circulating MMP[10]. Other effects of vitamin D are the suppression of fibroblasts proliferation and the increase in collagen production[9].

The purpose of this study is to evaluate serum concentrations of 25(OH)D patients with chronic hepatitis and to study the relationship between vitamin D level and fibrotic stage in patients with chronic viral hepatitis.

PATIENTS AND METHODS

Patients

From January 2011 to January 2012, patients with chronic HBV and HCV infection who attended the GI clinic, Ramathibodi Hospital (an 800-bed university hospital), Mahidol University, Bangkok, Thailand, were conducted. The diagnosis of HBV or HCV infection was based on the finding of seropositivety for either hepatitis B surface antigen (HBs Ag) or HCV RNA respectively. Exclusion criteria were (1) decompensated liver disease (Child-Pugh score B, C); (2) coinfection with HIV; (3) presence of other liver diseases; (4) hepatocellular carcinoma; (5) other autoimmune diseases; (6) other cancer; (7) chronic kidney disease; (8) metabolic disease associated with vitamin D and calcium metabolism (e.g. intestinal disease, bone disease, chronic infection,sarcoidosis); (9) history of immunomodulatory and /or immunosuppressive drugs; (10) current or previous treatment with antiviral therapy; and (11) current used mineral or vitamin D supplement. The present study was approved by the Ramathibodi Hospital Ethics Committee.

METHODS

Clinical and Laboratory Assessment

All patients had a detailed demographic and medical history taken, and underwent a through physical examination. Clinical and anthropometric data were collected at the time of liver biopsy. Body mass index was calculated on the basis of weight in kilograms and

height in meters. Waist circumference was measured at the midpoint between the lower border of the rib cage and the iliac crest. Laboratory tests include complete blood count (CBC), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), total bilirubin, total cholesterol, triglycerides, and prothrombin time (PT).

The analysis of serum 25(OH)D was performed using the LIAISON(r) 25OH vitamin D assay, a direct competitive chemiluminescence immunoassay (CLIA) for quantitative determination of total 25OH vitamin D. In accordance with the kit's instructions, a serum 25(OH)D concentration of 20 ng/mL was considered the threshold value for identifying low levels of vitamin D.

Histological analysis

Liver biopsy specimens were fixed, paraffin embedded and routinely stained with hematoxylin-eosin and trichrome stains. All biopsy specimens were carefully interpreted by pathologists, and were reported according to the METAVIR group scoring system. Pathologic findings were staged on a scale of F0 to F4 (F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = few septa, F3 = numerous septa without cirrhosis, F4 = cirrhosis). Histological activity, a measure of intensity of necroinflammatory lesions was graded as follow: A0 = no histological activity, A1 = mild activity, A2 = moderate activity, and A3 = severe activity.

Statistical analysis

Continuous variable with a normal distribution are shown as mean values (± standard deviation [SD]). Categorical data are shown as frequency and percentage. Continuous data were compared between the 2 groups using an independent t test or Mann-Whitney U test. The Chi-square test or the Fisher exact test was used for categorical data analysis. The incidence of vitamin D deficiency was compare between patient groups by using 2 × 2 contingency tables. Logistic regression analysis was used to measure the contribution of variables to the value of independent variables in a multivariate logistic regression. The odds ratio (OR) and its 95% confidence interval (CI) were estimated. All analyses were performed using Stata, version 12.0. A *p*-value of <0.05 was considered to be statistical significant.

Table 1. Baseline demographic, laboratory, and histological features of 81 patients with chronic hepatitis B and C.

Variable	Chronic hepatitis patients (n=81)
Mean age±SD (yrs.)	49 ± 11
Sex (%)	
Male/Female	33 (41)/48 (59)
Mean body mass index (kg/m^2)	24 ± 4
Waist circumference (cm)	85 ± 9
Hemoglobin (g/dL)	13.92 ± 3.14
Platelet count (x 10^3/mm^3)	204 ± 648
INR	1 ± 0.07
Alanine aminotransferase (IU/L)	108 ± 75
Alkalinephosphatase (IU/L)	93 ± 27
Gamma glutamyl transferase (IU/L)	96 ± 80
Cholesterol (mg/dL)	192 ± 37
Triglycerides (mg/dL)	102 ± 52
Mean 25(OH)D (ng/mL)	
CHB	21.07 ± 6.47
CHC	24.15 ± 9.15
Total	22.32 ± 7.77
Serum 25-hydroxyvitamin D < 20 ng/mL (n,%)	
No	47 (58)
Yes	34 (42)
Serum 25-hydroxyvitamin D < 20 ng/mL (n,%)	
CHB	23 (48)
CHC	11 (33)
Mean HBV-DNA (IU/mL x 10^3)	363 ± 124
Mean HCV-RNA (IU/mL x 10^3)	265 ± 186
Histology stage of fibrosis (Metavir score) (n,%)	
0	15 (17)
1	19 (22)
2	27 (31)
3	17 (20)
4	9 (10)
Grade of inflamation (n,%)	
0	21 (25)
1	42 (50)
2	16 (19)
3	5 (6)

IU, international units; HCV-RNA, hepatitis C virus ribonucleic acid; HBV-DNA, hepatitis B deoxyribonucleic acid. Data are given as mean and standard deviation or as number of cases (%).

RESULTS

Epidemiological, clinical, virological, biochemical, and histological characteristic of 81 Thai patients with CHB and CHC are summarized in Table 1. A total of 48 (59%) and 33 (41%) were CHB, and CHC, respectively. The mean BMI was 24 ± 4 kg/m^2. Biochemical laboratory showed mild elevation ALT and ALP. Mean vitamin D levels of all patients, CHB and CHC patients were 22.32 ± 7.77 ng/mL, 21.07± 6.47 ng/mL and 24.15 ± 9.15 ng/mL, respectively. Fourty two percent of the patients were found to have vitamin D deficiency which was defined as a serum concentration of 25(OH)D < 20 ng/mL. Twenty three (48%) and 11 (33%) of CHB and CHC patients were found to have vitamin D deficiency. One third of patients had fibrosis of at least F3 by Metavir score, with a small prevalence of moderate/severe fibrosis (grading F3-4). Two third of the cases had histological evidence of mild degree of fibrosis. Half of patient had mild inflamation of necroinflammatory activity. Only 5 (6%) of all patient were found to have severe necroinflammation.

Table 2, Figure1, and Figure 2 display correlation between mean serum concentration of 25(OH)D and fibrosis score. The mean serum concentration of 25(OH)D of CHB patients in F0-1, F2, F3-4 were 22.11 ± 6.88 ng/mL, 22.14 ± 6.51 ng/mL, 17.90 ± 4.93 ng/mL, respectively. The mean serum concentration of 25(OH)D of CHC patients in F0-1, F2, F3-4 were 23.35 ± 8.98 ng/mL, 22.70 ± 8.64 ng/mL and 25.89 ± 10.58 ng/mL, respectively. The mean serum concentration of 25(OH)D of total patients in F0-1, F2, F3-4 were 22.54 ± 7.55 ng/mL, 22.36 ± 7.27 ng/mL, 21.72 ± 8.93 ng/mL, respectively.

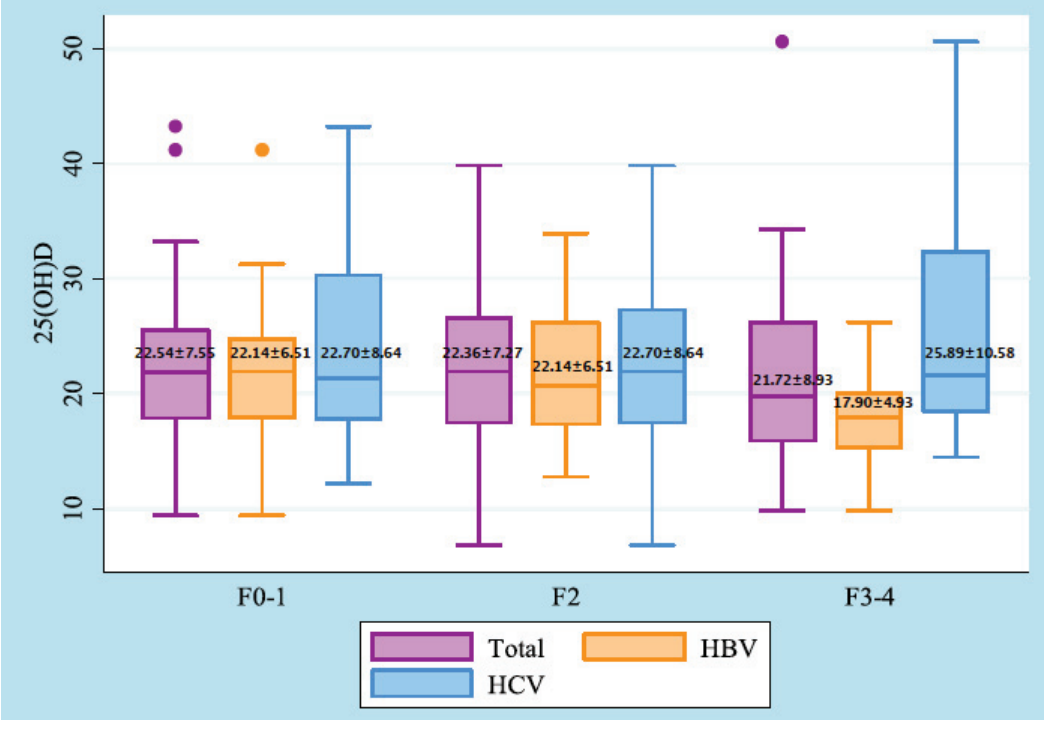

Figure 1. Distribution of 25(OH)D serum level according to different stage of fibrosis.

Table 2. Vitamin D deficiency in patients with chronic hepatitis B and C.

		Chronic viral hepatitis		Fibrosis score		
25(OH)D (ng/mL)	All hepatitis pateint (n,%)	CHB (n,%)	CHC (n,%)	F0-1 (n,%)	F2 (n,%)	F3-4 (n,%)
<20	34 (42)	23 (48)	11 (33)	12 (37.5)	10 (40)	12 (52)
>20	47 (58)	25 (52)	22 (67)	20 (62.5)	15 (60)	11 (48)

Table 3. Multivariate logistic regression analysis impact of fibrosis with vitamin D deficiency.

Fibrosis stage	25(OH)D<20 ng/mL (n,%)	Odds ratio	95%CI	*p*-value
F0-1	12 (37.5%)	1		
F2	10 (40%)	1.11	0.380, 3.251	0.847
F3-4	12 (52%)	1.82	0.631, 5.391	0.613

Table 3 displays the incidence of 25(OH)D_3 deficiency among patients with CHB and CHC which tended to correlate with fibrosis stage (37.5%, 40% and 52.1% in metavir fibrosis score F0-1, F2, F3-4 respectively, p=0.529). From forward stepwise logistic regression in Table 4, vitamin D deficiency tended to be associated with significant fibrosis (F2 fibrosis stage) of CHB and CHC (OR, 1.11; 95% CI, 0.379-3.250; p=0.847) and severe fibrosis (F3-4 fibrosis stage) of CHB and CHC (OR, 1.81; 95% CI,0.613-5.39; p= 0.281).

DISCUSSION

Several studies propose factors correlated with cirrhosis. Vitamin D is one of them. Previous studies have reported results about serum concentrations of 25(OH)D and its relationship with the severity of liver disease. Recently, Petta et al showed the first evidence that low serum 25(OH)D levels, together with known risk factors for fibrosis severity, such as older age, low cholesterol levels, and high necroinflammatory activity were independently associated with the presence of severe fibrosis (Metavir scoring system F3-4) in CHC[4]. Lange et al found the high incidence of severe vitamin D deficiency in patients with CHC and the association of vitamin D deficiency with the degree of fibrosis[11].

Our study represents the first study of vitamin D status in CHB and CHC infection of Thai population. The major results were (1) a high incidence of severe vitamin D deficiency in patient with CHB and CHC (2) a trend of association between serum 25(OH)D level and severity of fibrosis.

The first large scale study of vitamin D status in the Thai population was reported by La-or Chailurkit et al. They reported the comparison of mean serum 25(OH)D levels between gender by age, municipal area, BMI in Thai population[12]. Our data revealed low serum 25(OH)D level in Thai patients with CHB and CHC infection comparing to the results of the latter study, 22.32 ± 7.77 ng/mL vs 32.29 ±0.4 ng/mL in age and sex match. Chronic viral hepatitis is able to interfere with several cellular pathway and role of immunoregulatory properties of vitamin D. The interaction with VDR protecting against oxidative stress production can influence the migration, proliferation, and gene expression of fibroblasts and reduces the inflammatory and fibrogenic activity of liver stellate cells. Future studies are required to establish the exact underlying mechanism[7-9].

Several trials have reported an association between vitamin D deficiency and liver fibrosis in patient with various caused of liver disease including CHC[4,11]. The association between viamin D deficiency and degree of liver fibrosis was suggested by our study. Our data revealed that 25(OH)D deficiency tended to have the correlation with advance fibrosis stage (37.5%, 40% and 52.1% in metavir fibrosis score F0-1, F2, F3-4 respectively, p=0.529).

The main limitation of this study was the small number of subjects. The study design is cross-sectional study which is unable to dissect the temporal relationship between 25(OH)D level and fibrosis. Another limitation of this study is the lack of data on the potential confounders that may influence the level of 25(OH)D, such as dietary intake and the prevalence of osteoporosis.

In conclusion our study reveals that vitamin D deficiency is highly prevalent in patients with CHB and CHC. The 25(OH)D serum levels deficiency have a trend for correlation with advance fibrosis stage. Further studies are required to confirm this finding.

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