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Original Article

Serum 25-hydroxyvitamin D status in pregnant women with chronic hepatitis B virus infection

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Abstract

Introduction: Maternal 25-hydroxyvitamin D [25(OH)D] deficiency has a negative influence on the health of the mother and the developing fetus. The aim of this study was to assess serum 25(OH)D status and its relationship to virologic and biochemical parameters in pregnant women with chronic hepatitis B virus (HBV) infection.

Methodology: Serum 25(OH)D levels among 142 pregnant women with chronic HBV infection and 251 healthy pregnant women were measured using enzyme-linked immunosorbent assay.

Results: The mean ± SD values for serum 25(OH)D levels were 13.63 ± 5.5 ng/mL in healthy pregnant women and 12.05 ± 3.3 ng/mL in pregnant women with chronic HBV infection ($p < 0.01$). Serum 25(OH)D levels were associated with seasonal variation in healthy pregnant women ($p = 0.01$); however, similar results were not observed in pregnant women with chronic HBV infection ($p = 0.10$). Furthermore, multivariate analysis indicated that only ALT level was independently associated with severe vitamin D deficiency ($p = 0.01$). A significant positive correlation was found between serum 25(OH)D level and ALT level in pregnant women with chronic HBV infection ($r = 0.32$; $p < 0.001$).

Conclusions: Vitamin D levels were lower in pregnant women with chronic HBV infection compared with healthy pregnant women. Vitamin D supplementation can be routinely recommended for pregnant women in China.

Key words: 25-hydroxyvitamin D; pregnancy; hepatitis B virus; infection.

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Introduction

Vitamin D is a fat-soluble vitamin and mainly made from 7-dehydrocholesterol in the skin by exposure to ultraviolet light. After undergoing the activation process of 25-hydroxylation in the liver and 1α-hydroxylation in the kidneys, vitamin D changes into the physiologically active metabolite, 1α,25-dihydroxyvitamin D [1α,25$(OH)_2$D], which expresses its biological activity through binding to and modulation of the vitamin D receptor. In these vitamin D metabolites, the half-life of vitamin D and 1α,25$(OH)_2$D is significantly shorter than 25-hydroxyvitamin D [25(OH)D] [1-3]. In addition, hepatic production of 25(OH)D is not significantly regulated and is primarily dependent on substrate concentration. For these reasons, serum 25(OH)D levels have been used to assess an individual's vitamin D status.

Maternal vitamin D status has an important effect on the health of the mother and fetus. Previous studies have indicated that vitamin D deficiency in pregnant women is significantly associated with increased risk of preeclampsia, gestational diabetes, preterm birth, and low birthweight [4-7]. Additionally, vitamin D supplementation could contribute to a reduced risk of preeclampsia and enhance weight gain and nutritional status in pregnant women [8,9]. Thus, understanding vitamin D status in a wide variety of pregnant women would be helpful in preventing the occurrence of pregnancy complications and adverse pregnancy outcomes. However, most studies have focused only on the vitamin D status of healthy pregnant women and pregnant women suffering pregnancy complications and adverse outcomes. Little published information is available regarding epidemiological data on vitamin D status in pregnant women with chronic hepatitis B virus (HBV) infection. Therefore, this case-control study was

conducted to assess vitamin D status and its relationship to virologic and biochemical parameters in pregnant women with chronic HBV infection.

Methodology

Study population

A total of 142 treatment-naive pregnant women with chronic HBV infection and 251 healthy pregnant women participated in the case-control study. Pregnant women with any renal illness, malabsorption syndrome, medications (current and past), and vitamin D supplementation were excluded from the study. All pregnant women lived in Jiangsu Province and were recruited from the Second Affiliated Hospital of the Southeast University, Nanjing, Jiangsu, during the winter of 2013 and the summer of 2014.

Ethics statement

All participants gave written informed consent. The study design and protocol were approved by the ethics committee of the Second Affiliated Hospital of the Southeast University, Nanjing, Jiangsu. Methods were carried out in accordance with the approved guidelines.

Serum extraction and determination of serum 25(OH)D level

Serum samples were obtained and stored at -80°C at the same time virologic and biochemical parameters were determined. Serum 25(OH)D level was quantified by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (25-Hydroxy Vitamin D EIA kit, Immunodiagnostic Systems, Boldon, UK). Vitamin D deficiency and severe vitamin D deficiency were defined as serum 25(OH)D level ≤ 20 ng/mL and ≤ 10 ng/mL, respectively.

Measurement of virologic and biochemical parameters

Serum HBsAg levels were determined using the Abbott Architect HBsAg QT assay (Abbott Diagnostics, Chicago, USA), following the manufacturer's instructions. The range of the Architects assay is 0.05–250 IU/mL, and an automatic 1:500 dilution is needed for higher levels (> 250 IU/mL). Serum HBV DNA levels were quantified by a determination kit for hepatitis B virus DNA (Life River, Shanghai, China) with a detection range of 5×10^2–1×10^8 IU/mL. For samples with HBV DNA > 10^8 IU/mL, the HBV DNA assay was repeated after a dilution of 1:1,000. HBeAg status was determined by ELISA (Abbott Laboratories, Chicago, USA). Biochemical parameters were measured with automatic biochemical analyzer (Roche, Basel, Switzerland).

Collection of epidemiological, virologic, and biochemical data

A trained investigator collected epidemiological data including age, body mass index (BMI), parity, gestation week, and season of blood test by personal interview. Two investigators independently extracted virologic and biochemical data from electronic medical records. Any disagreements were resolved through discussion.

Statistical analysis

Statistical analysis of the data was carried out using SPSS statistical software package version 19.0. Continuous variables and categorical variables are presented as mean ± standard deviation (SD) and number (%), respectively. Proportions were compared using the Chi-square test. Group means were compared using the independent samples t test. A logistic regression model was used to assess the change of serum 25(OH)D levels between HBV-infected pregnant women and controls, the relationship of seasonal variation (winter and summer) with serum 25(OH)D levels, and the association between serum 25(OH)D status [25(OH)D ≤ 10 ng/mL and 25(OH)D > 10 ng/mL] and virologic and biochemical parameters. After univariate analysis, only variables associated with the dependent variable were included in the multivariate logistic regression analysis. Stepwise logistic regression analysis with a forward approach was performed to identify independent variables of severe vitamin D deficiency. Furthermore, correlation between serum 25(OH)D levels and ALT levels was assessed by Spearman's correlation analysis. All $p < 0.05$ was considered statistically significant.

Results

Serum 25(OH)D levels among HBV-infected pregnant women and controls

The baseline characteristics of the subjects are summarized in Table 1. A total of 142 HBV-infected pregnant women and 251 control subjects were included in the study. Among them, there were 84 HBV-infected pregnant women and 142 control subjects recruited in winter, and 58 HBV-infected pregnant women and 109 control subjects recruited in summer. No statistically significant differences were found between HBV-infected pregnant women and control subjects in terms of age, BMI, parity, gestational weeks, and blood test season, suggesting that the frequency matching was

Table 1. Baseline characteristics among hepatitis B virus (HBV)-infected pregnant women **and controls.**

Characteristic	HBV-infected pregnant women (n = 142)	Controls (n = 251)	P
Age (years)	25.8 ± 3.5	26.5 ± 3.8	0.07
Body mass index (kg/m^2)	23.8 ± 3.2	23.2 ± 3.2	0.10
Parity			
0	117 (82.4)	196 (78.1)	0.31
1	25 (17.6)	55 (21.9)	
Gestation (weeks)	24.3 ± 7.7	24.5 ± 7.9	0.85
Season of blood test			
Winter[a]	84 (59.2)	142 (56.6)	0.62
Summer[b]	58 (40.8)	109 (43.4)	
HBV DNA level			
≥ 2.7 $\log_{10}$ IU/mL	121 (85.2)	—	
< 2.7 $\log_{10}$ IU/mL	21 (14.8)	—	
HBsAg, $\log_{10}$ IU/mL	3.8 ± 0.9	—	
HBeAg			
Positive	110 (77.5)	—	
Negative	32 (22.5)	—	

[a] December to February; [b] June to August; The values of HBV DNA level were cut off based on a detection limit of 5×10^2 IU/mL (2.7 $\log_{10}$ IU/mL)

adequate. Vitamin D statuses in HBV-infected pregnant women and control subjects are shown in Table 2. Serum 25(OH)D levels in HBV-infected pregnant women were significantly lower than those in control subjects (HBV-infected pregnant women vs. control subjects: 12.05 ± 3.3 vs. 13.63 ± 5.5, p < 0.01). Similar results were also observed in stratified analysis based on seasons (HBV-infected pregnant women vs. control subjects in winter: 11.70 ± 2.8 vs. 12.74 ± 5.0, p = 0.05; HBV-infected pregnant women vs. control subjects in summer: 12.54 ± 3.8 vs. 14.80 ± 6.0, p = 0.01). Additionally, the prevalence of vitamin D deficiency was also different between HBV-infected pregnant women and control subjects. The proportions of vitamin D deficiency were significantly higher in HBV-infected pregnant women compared to control subjects (HBV-infected pregnant women vs. control subjects: 98.6% vs. 85.3%, p < 0.01). Similar results were also observed in stratified analysis based on seasons (HBV-infected pregnant women vs. control subjects in winter: 100% vs. 88.7%, p = 0.01; HBV-infected pregnant women vs. control subjects in summer: 96.6% vs. 80.7%, p = 0.01).

Figure 1. Association between serum 25(OH)D levels and seasonal variation (p values were adjusted for age, BMI, parity, and gestation).

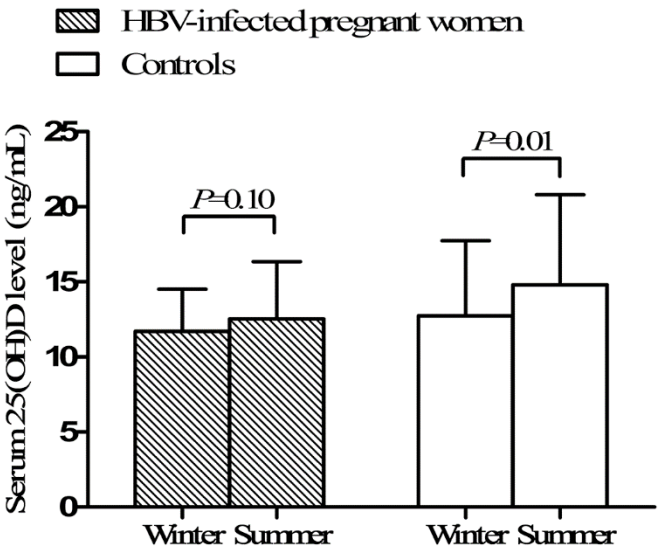

Table 2. Vitamin D status in hepatitis B virus (HBV)-infected pregnant women and controls

	All			Winter			Summer		
	HBV-infected pregnant women (n = 142)	Controls (n = 251)	P[a]	HBV-infected pregnant women (n = 84)	Controls (n = 142)	P[a]	HBV-infected pregnant women (n = 58)	Controls (n = 109)	P[a]
Vitamin D level, ng/mL	12.05 ± 3.3	13.63 ± 5.5	< 0.01	11.70 ± 2.8	12.74 ± 5.0	0.05	12.54 ± 3.8	14.80 ± 6.0	0.01
25(OH)D > 20 ng/mL	2 (1.4)	37 (14.7)	< 0.01	0 (0)	16 (11.3)	0.01	2 (3.4)	21 (19.3)	0.01
25(OH)D ≤ 20 ng/mL	140 (98.6)	214 (85.3)		84 (100)	126 (88.7)		56 (96.6)	88 (80.7)	

[a] Adjusted for age, body mass index, parity, gestation, and season of blood test.

Serum 25(OH)D levels and seasonal variation

As shown in Figure 1, serum 25(OH)D levels were associated with seasonal variation in healthy pregnant women. Healthy pregnant women evaluated in summer had higher serum 25(OH)D levels than did healthy pregnant women evaluated in winter (p = 0.01). However, a significant seasonal variation of serum 25(OH)D levels was not observed in pregnant women with chronic HBV infection (p = 0.10).

Association between 25(OH)D status and virologic and biochemical parameters

Characteristics of HBV-infected pregnant women, stratified by vitamin D status [25(OH)D ≤ 10 ng/mL and 25(OH)D > 10 ng/mL], are summarized in Table 3. The results of virologic parameters showed that HBV DNA level, HBsAg level, and HBeAg status were not significantly different between HBV-infected pregnant women with 25(OH)D ≤ 10 ng/mL and those with 25(OH)D > 10 ng/mL (p = 0.36, 0.67, 0.44, respectively); however, the levels of alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transpeptidase (GGT), and mitochondrial-glutamic oxaloacetic transaminase (MAST) in biochemical parameters were significantly different between the two groups (p = 0.02, 0.04, 0.05, 0.04, respectively). The levels of ALT, AST, GGT, and MAST were lower in HBV-infected pregnant women with 25(OH)D ≤ 10 ng/mL compared to those with 25(OH)D > 10 ng/mL. In the multivariate analysis, only ALT level was independently associated with severe vitamin D deficiency (p = 0.01).

Correlation between serum 25(OH)D levels and ALT levels

Considering that ALT level was independently associated with severe vitamin D deficiency in the multivariate analysis, the correlation between serum 25(OH)D levels and ALT levels was further assessed (Figure 2). Results showed a significantly positive

Figure 2. Correlation between serum 25(OH)D levels and ALT levels in HBV-infected pregnant women (n = 142, r = 0.32, p < 0.001).

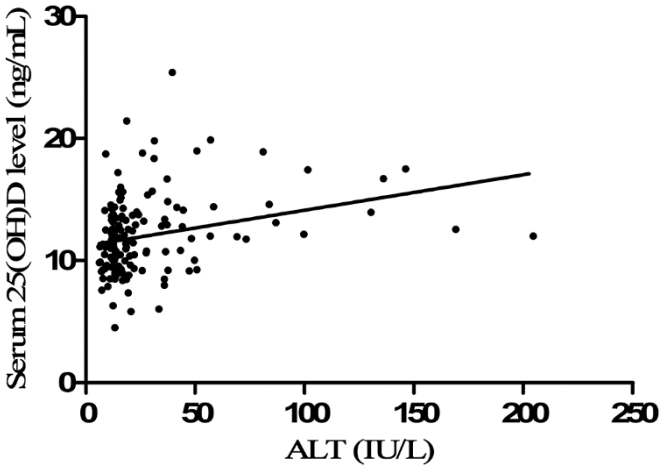

Table 3. Characteristics of hepatitis B virus (HBV)-infected pregnant women according to serum vitamin D status

Characteristic	25(OH)D ≤ 10 ng/mL	25(OH)D > 10 ng/mL	P[a]	P[b]
Number	41 (28.9)	101 (71.1)		
Virologic parameters				
HBV DNA, log_{10} IU/mL	6.83 ± 1.7	7.13 ± 1.5	0.36	
HBsAg, log_{10} IU/mL	3.80 ± 1.0	3.87 ± 0.9	0.67	
HBeAg				
Positive	30 (73.2)	80 (79.2)	0.44	
Negative	11 (26.8)	21 (20.8)		
Biochemical parameters				
Total bilirubin, umol/L	8.94 ± 3.0	8.05 ± 2.4	0.07	
Indirect bilirubin, umol/L	6.74 ± 2.5	5.94 ± 2.2	0.07	
Direct bilirubin, umol/L	2.20 ± 1.4	2.11 ± 1.3	0.71	
Total protein, g/L	68.90 ± 4.5	68.48 ± 4.7	0.62	
Albumin, g/L	38.71 ± 4.1	38.78 ± 3.7	0.92	
Globulin, g/L	30.13 ± 2.6	29.71 ± 3.3	0.47	
Albumin/globulin	1.30 ± 0.2	1.32 ± 0.2	0.45	
ALT, IU/L	17.59 ± 10.8	32.69 ± 35.6	0.02	0.01
AST, IU/L	20.30 ± 8.5	29.30 ± 25.3	0.04	
GGT, U/L	11.43 ± 4.9	13.94 ± 7.2	0.05	
MAST, U/L	3.30 ± 1.6	4.64 ± 3.6	0.04	
Cholinesterase, U/L	6,681.61 ± 1,757.9	6,281.44 ± 1,318.2	0.14	
Lactate dehydrogenase, IU/L	188.61 ± 32.4	187.24 ± 33.2	0.82	

ALT: alanine transaminase; AST: glutamic-oxaloacetic transaminase; GGT: gamma-glutamyl transpeptidase; MAST: mitochondrial-glutamic oxaloacetic transaminase; [a] Univariate analysis; [b] multivariate logistic regression analysis.

correlation between serum 25(OH)D levels and ALT levels ($r = 0.32$; $p < 0.001$).

Discussion

It is well known that the occurrence of pregnancy complications and adverse outcomes, such as gestational diabetes mellitus, preeclampsia, preterm birth, and low birthweight, has a negative influence on the health of the mother and the developing fetus. However, the pathogenesis of pregnancy complications and adverse outcomes is still not completely elucidated. Recently, some studies showed that the susceptibility of pregnancy complications and adverse outcomes was higher in HBsAg-positive pregnant women compared to HBsAg-negative pregnant women [10-12]. Therefore, it is urgent for HBV-infected pregnant women to find risk factors associated with pregnancy complications and adverse outcomes. For most pregnant women, vitamin D deficiency was associated with an increased risk of pregnancy complications and adverse outcomes [4-7]. In the present study, we found that vitamin D levels in HBV-infected pregnant women were significantly lower than those in healthy pregnant women, which could partially explain the higher prevalence of pregnancy complications and adverse outcomes among HBV-infected pregnant women. Furthermore, the vast majority of pregnant women had 25(OH)D levels ≤ 20 ng/mL. Based on these findings, vitamin D supplementation can be routinely recommended to pregnant women in China. Interestingly, the associations between serum 25(OH)D levels and seasonal variation were observed only in healthy pregnant women, but not in HBV-infected pregnant women. This phenomenon suggests that chronic HBV infection might have a negative effect on endogenous vitamin D synthesis via sunlight exposure. Univariate analysis by virologic parameters showed that HBV DNA level, HBsAg level, and HBeAg status were not associated with vitamin D status; however, univariate analysis based on biochemical parameters suggested that the levels of ALT, AST, GGT, and MAST were significantly associated with vitamin D status. In multivariate analysis, we found that ALT levels were independently associated with severe vitamin D deficiency among HBV-infected pregnant women. Furthermore, Spearman's correlation analysis showed a significantly positive correlation between serum 25(OH)D levels and ALT levels in HBV-infected pregnant women. HBV is a non-cytopathogenic hepadnavirus, and liver damage in HBV patients is closely associated with host immune response induced by HBV [13]. Recent studies have indicated that vitamin D plays a vital role in modulating the host immune response to infection. For example, vitamin D can stimulate production of antimicrobial peptides (AMPs) such as α-defensins, β-defensins, and cathelicidin [14,15]. Aside from their direct microbicidal role, AMPs modulate many other immune processes, including mast cell degranulation, cell differentiation, vascular permeability, and the process of antigen presentation [16,17]. Therefore, vitamin D may contribute to HBV-associated liver injury by regulating the innate and/or adaptive immune system. However, correlation does imply causation. Previous studies have suggested that vitamin D inhibits the development of Th1 and increases the number of Th2 cells, which limits tissue damage from the inflammatory response [18]. Thus, ALT may act as one of the rescue signals and stimulate vitamin D expression to reduce liver injury.

Some limitations of the present study should be acknowledged. Firstly, sample sizes included in the study were not as large as we had wanted, which may have resulted in a reduction of the statistical power. Furthermore, all subjects were recruited only in winter and summer. Although the biggest change in sunlight intensity was observed between winter and summer, which were the most representative seasons, a study that investigates the association between vitamin D levels of HBV-infected pregnant women and seasonal variation should be performed in all seasons, which would help to fully understand the relationship between two variables. Despite these above limitations, our study had some strong advantages. To our knowledge, this is the first study to assess vitamin D status in HBV-infected pregnant women. In addition, this study comprehensively and systematically investigated the relationship of vitamin D status with virologic and biochemical parameters in HBV-infected pregnant women.

Conclusions

Our study suggested that serum 25(OH)D levels were lower in pregnant women with chronic HBV infection compared to those in healthy pregnant women, and serum 25(OH)D levels were positively correlated with ALT levels in pregnant women with chronic HBV infection.

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