Serum vitamin D levels in treatment-naïve chronic hepatitis B patients

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ABSTRACT

Background and Objectives: According to the demographic health survey conducted in 2015, Egypt had 10% documented prevalence of anti-HBc positive patients aged 1-59 and 1% viremic patients amongst the population in the same age group, with a domination of genotype D. Several studies claimed the possible role of vitamin D deficiency in hepatitis B virus (HBV) replication and disease progression. Patients and Methods: Serum vitamin D levels [25(OH) D3] were assessed in 96 HBeAg negative non-cirrhotic chronic HBV patients and 25 healthy subjects classified as following: Group I: 48 chronic HBV patients with persistently normal ALT levels and HBV DNA level < 2000 IU/mL for ≥ 6 months; Group II: 48 chronic HBV patients with CHB with persistently elevated ALT and HBV DNA level ≥ 2000 IU/mL for ≥ 6 months; and Group III: 25 apparently healthy subjects with normal liver enzymes and negative hepatitis viral markers were taken as the control group. Results: Vitamin D was much more deficient in group II than in group I and group III being 11.55 ± 3.97 ng/mL, 15.03 ± 3.45 , 27.00 ± 6.76 ng/mL (P < 0.001), respectively, and a strong negative correlation was observed between vitamin D levels and HBV DNA levels (P = 0.043) in groups I and II. Conclusion: The current study showed high HBV DNA replication in patients with vitamin D deficiency suggesting the antimicrobial immunomodulatory role of vitamin D.

Key words: Egyptian, hepatitis B virus, vitamin D

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INTRODUCTION

Egypt has one of the highest burdens of viral hepatitis globally. In 2015, a demographic health survey (DHS) documented the prevalence of anti-HBc in 10% of the Egyptian population between the age group 1-59 years, with slight male predominance and great variation in age distribution (less than 1% among children aged 1-14 years, 43% among adults aged 55-59 years). In this survey, the patients with active hepatitis B virus (HBV) infection and positive viremia represent 1% of the population in the same age group. [1] Genotype D is the major infecting genotype in Egypt representing more than 85% of those with chronic HBV.[2]

The liver is a major site for vitamin D synthesis, where 25-hydroxylation occurs, and a large portion vitamin D binding protein is manufactured. Due to its immunomodulatory role, there is growing evidence about the interrelationship between vitamin D and different chronic liver diseases in different stages.[3] Different studies have shown a wide, universal agreement of vitamin D deficiency in patients with chronic hepatitis C virus (HCV) infection, [4,5] chronic HBV, [6,7] nonalcoholic steatohepatitis (NASH)[8] and hepatocellular carcinoma (HCC)[9] with contradictory reports about the impact of vitamin D deficiency on disease pathology and progression.

Although most countries in Africa are sunny throughout the year, but still vitamin D deficiency is a major problem in most African countries, [10] particularly Egypt, which suffers from this problem in both diseased[11] and healthy people. [12,13].

The current study was designed to assess the serum levels of vitamin D [25(OH) D3] in the treatment naïve chronic HBV patients and its impact on the disease status.

PATIENTS AND METHODS

The study was reviewed and approved by an independent ethics committee and conducted in accordance with the declaration of Helsinki and good clinical practice guidelines. All authors had access to the study data, and they reviewed and approved the final manuscript. All enrolled patients provided written, informed consent prior to the start of the study. It was a prospective study in which 121 subjects were enrolled between July 2015 and February 2016. A total of 96 HBeAg negative chronic HBV patients and 25 healthy subjects were recruited and sub-classified into 3 groups: Group I: 48 chronic HBV patients with persistently normal ALT level and HBV DNA level $< 2000 \text{ IU/mL for } \ge 6$ months; Group II: 48 chronic HBV patients with CHB with persistently elevated ALT and HBV DNA level ≥ 2000 IU/mL for \geq 6 months; **Group III:** 25 apparently healthy subjects with normal liver enzymes and negative hepatitis viral markers were taken as the control group.

Inclusion criteria

Adults of both genders ≥ 18 years old with proven chronic HBV infection (defined as HBsAg+ and Positive HBV DNA PCR [polymerase chain reaction] for ≥ 6 months) were included. All included patients were HBV genotype D.

Exclusion criteria

- 1. Co-infection with HCV, hepatitis D virus (HDV), and human immunodeficiency virus (HIV);
- 2. Advanced liver fibrosis (fibroscan ≥ 12.5 kPa, liver biopsy ≥ F3 by Metavir score)
- 3. HCC or decompensated liver cirrhosis;
- 4. Those who were receiving medications known to affect vitamin D3 level or metabolism (calcium, vitamin D supplementation, oestrogen, alendronate, isonicotinic acid hydrazine [INH], Thiazide diuretics, long-term antacids, calcium channel blockers, cholestyramine, anticonvulsants, orlistat);
- 5. Renal, or parathyroid disease;
- 6. Chronic intestinal disease.

Laboratory investigations

We performed laboratory study as detailed below:

1. Full routine laboratory investigations;

- 2.HBV DNA measurement by real time PCR (Roche): Serum HBV-DNA was measured by use of COBAS AmpliPrep/COBAS TaqMan with detection limit of 12 IU/mL. HBV-DNA levels were expressed in IU/mL;
- 3. Serum vitamin D level [25(OH)D3]: by using enzymelinked immunosorbent assay (ELISA) kit, we measured serum 25(OH) vitamin D, the most stable circulating form of this molecule using DRG Assay quantitative determination of 25-OH Vitamin D in plasma and serum (DRG International Inc., USA);
- 4. To avoid seasonal variations of vitamin D level, the blood samples of the whole study populations were withdrawn in the same month (February 2016).

Statistical analysis

The collected data were tabulated and analysed using SPSS version 16 software (Spss Inc, Chicago, ILL Company) and MedCalc software. Categorical data were presented as number and percentages while quantitative data were expressed as mean \pm standard deviation, median and range. Chi square test (χ^2), or Fisher's exact test (FET) were used to analyse categorical variables. Quantitative data were tested for normality using Kolmogorov–Smirnov test, using Student $\mathscr T$ and if normally distributed, or Mann Whitney U test, Kruskal Wallis test and Spearman's correlation coefficient (rho) if not normally distributed. The accepted level of significance in this work was stated at 0.05 (P < 0.05 was considered significant).

RESULTS

In the current study, the patients' enrolment and categorization was done on the basis of follow up of liver enzymes (once in 3 months) and HBV viral load (once in 3 months); the patients with normal ALT levels and HBV viral load < 2000 IU/mL in 2 consequent visits were allocated to group I of the study, while those with elevated ALT and HBV viral load ≥ 2000 IU/mL in 2 consequent visits were allocated to group II. The patients with fluctuating ALT levels or viral load were not included in the trial. All enrolled patients were treatment naïve and had a negative history of receiving any specific antiviral drugs before inclusion in the study. The results of vitamin D level assay were interpreted as deficiency level (< 20 ng/mL), insufficiency level (20-30 ng/mL) and normal level (> 30 ng/mL)[14]. The 3 groups had similar values for age, BMI and sex distribution with significant higher levels of AST, ALT, AFP and HBV DNA and lower levels of albumin were observed in group II compared to other groups (P < 0.001), as shown in Table 1.

Table 2 describes the noticed levels of vitamin D insufficiency in the control group compared to the clear deficiency noticed in both study groups (I, II) cumulatively

with (P < 0.001), or even separately as in Table 3, Figure 1 which showed a descending level of vitamin D for the control group (III) followed by the inactive chronic HBV group (I), then the lowest level in the active chronic HBV group (II) (P < 0.001).

Table 4, Figures 2, 3 showed a strong negative correlation between vitamin D levels and the degree of HBV viremia, the high DNA replication occurs in those with more deficient vitamin D levels and vice versa in groups I, II (P = 0.043, P < 0.001) respectively. Positive correlation between BMI and vitamin D levels was noticed in group I, II (P = 0.024, P < 0.001) respectively. Also, a positive correlation was noticed in group II between vitamin D levels with albumin and platelet count (P = 0.015, P = 0.002) respectively.

DISCUSSION

The influence of vitamin D deficiency on augmentation of many chronic liver diseases had reached to a near evidence. Many clinical trials and meta-analyses have strongly linked vitamin D deficiency with liver fibrosis progression, regardless the aetiology of chronic liver disease, with unclear causality relationship whether liver morbidity, affects vitamin D synthesis or the vitamin deficiency and is the contributor in the development of liver pathology. [15]

The current study tested the hypothesis of vitamin D deficiency and HBV replication in 96 HBeAg negative non-cirrhotic chronic HBV patients; in this study, the nullification of many factors that could negatively implicate the harmony of the results was maximally done, like inclusion of patients without significant fibrosis, HBeAg negative, matched age and sex, vitamin D sample withdrawal in the same month, and so on.

Egypt has a problem of vitamin D deficiency that seems

	Group I	Group II	Group III	P value
	(n=48)	(n=48)	(n=25)	
Age (years)	40.25 ± 9.46	38.22 ± 9.64	38.4 ± 10.42	0.58
Gender (males/females), n(%)	30(62)/18(38)	31(64)/17(36)	15(60)/10(40)	0.45
BMI (kg/m²)	30.55 ± 4.63	30.65 ± 4.45	28.8 ± 3.12	0.16
AST (IU/L)	23.7 ± 10.54	43.7 ± 22.4	29.7 ± 8.40	< 0.001
ALT (IU/L)	20.7 ± 9.5	53.1 ± 27.68	27.5 ± 6.13	< 0.001
Bilirubin (mg/dL)	0.81 ± 0.26	0.79 ± 0.27	0.75 ± 0.20	0.69
Albumin (g/dL)	4.34 ± 0.33	4.09 ± 0.49	4.49 ± 0.46	< 0.001
PT (s)	13.2 ± 0.83	13.3 ± 0.75	13.0 ± 0.75	0.5
Haemoglobin (g/dL)	13.4 ± 2.03	13.6 ± 1.83	12.7 ± 1.06	0.14
WBCs (109/L)	5.92 ± 1.66	6.21 ± 1.49	6.74 ± 1.88	0.13
Platelets (10 ⁹ /L)	210.4 ± 56.61	201.6 ± 45.32	226.2 ± 83.35	0.24
AFP (ng/mL)	2.88 ± 01.31	3.40 ± 1.860	1.21 ± 1.03	< 0.001
HBV DNA (IU/mL)	673.98 ± 572.27	2925103.8 ± 14888722.3	-	< 0.001

BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; PT: prothrombin time; WBCs: white blood cell counts; AFP: alpha-fetoprotein.

Table 2: Comparison between serum vitamin D levels in the study groups (patients vs. control)				
Group	n	Serum vitamin D (ng/mL)	<i>P</i> value	
		Mean ± SD		
Patients (Group I, II)	96	13.3 ± 4.1	< 0.001	
Control (Group III)	25	27.00 ± 6.76		

Table 3: Comparison between serum vitamin D levels in the 3 study groups					
Groups	n	Serum vitamin D (ng/mL)	P value		
		Mean ± SD			
I	48	15.03 ± 3.45			
II	48	11.55 ± 3.97	< 0.001		
III	25	27.00 ± 6.76			

Table 4: Correlation between serum vitamin D level and assessed variables in the studied cases groups:

	Serum Vitamin D					
Variables	$\overline{\text{Group I } (n = 48)}$		Group II (<i>n</i> = 48)			
	\overline{r}	Р	r	Р		
Age	- 0.01	0.95	0.07	0.64		
ВМІ	0.326	0.024	0.497	< 0.001		
ALT	- 0.048	0.74	- 0.011	0.94		
AST	- 0.006	0.97	- 0.123	0.41		
Total bilirubin	- 0.046	0.75	- 0.276	0.058		
Albumin	0.131	0.37	0.350	0.015		
INR	- 0.112	0.45	- 0.265	0.07		
Haemoglobin	0.023	0.87	0.148	0.32		
WBCs	0.04	0.78	0.09	0.55		
Platelets	0.05	0.75	0.467	0.002		
AFP	- 0.091	0.54	0.217	0.14		
HBV-DNA-PCR	- 0.293	0.043	- 0.114	< 0.001		

BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; INR: international normalized ratio; WBCs: white blood cell counts; AFP: alpha-fetoprotein; PCR: polymerase chain reaction.

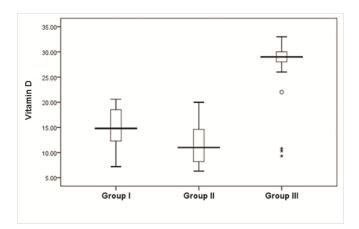


Figure 1: Serum vitamin D level in the 3 study groups

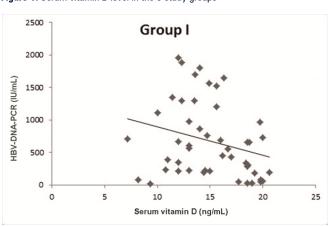


Figure 2: Correlation between serum vitamin D level and HBV-DNA-PCR in group I

to be on a national base and may not be directly related to certain diseases. Many published data suggest multiple possible contributing factors, including dark skin colour, dietary calcium deficiency and inadequate sun exposure. [13] The data explained the relatively low levels of vitamin D in the control group.

On the other hand, both chronic HBV groups (I, II) showed reduced levels compared to the control group. In group (I), which represented the inactive HBV disease, the level of vitamin D was significantly higher than group (II), which represented the active HBV infection, suggesting the immunomodulatory effect of vitamin D and its role

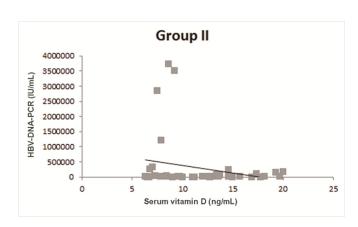


Figure 3: Correlation between serum vitamin D level and HBV-DNA-PCR in group II

as anti-microbial agent. [6] This could explain the high viral replication in group (II) compared to group (I) and its subsequent impact on the elevated AST, ALT and AFP levels and relatively reduced levels of albumin in group (II) compared to group (I). The absence of liver cirrhosis and other confounding factors in these groups confirms to a great extent the role of vitamin D in HBV replication and its ability to transform its status from an inactive to an active disease.

Our study observed the patients in only 2 stages of the disease: the inactive stage and the stage of chronic hepatitis. Yet, Hoan and his colleagues described more frequent vitamin D deficiency in post HBV cirrhotic patients and those with HBV related HCC than those with chronic active hepatitis. ^[16] Patients with positive HBeAg were not included in this study, but few studies reported more reduction in vitamin D levels in patients with positive HBeAg than those who were negative. ^[7,17]

In our study, although vitamin D levels had a strong negative correlation with HBV DNA levels, yet it did not show any correlation with the degree of necroinflammation represented by AST, ALT levels, in contradiction to some studies. [17] Yet the inflammatory changes in liver histology sometimes did not match with the level of liver enzymes.

In conclusion, the current study highlights the impact of vitamin D deficiency on HBV replication in particular, and its role in disease activity. Meanwhile, it is recommended to study vitamin D status in all HBV stages and to study the impact of vitamin D replacement and correction on the disease progression or regression in different stages.

Conflict of Interest

All authors report no conflicts of interest.

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