Original Article

Impact of Chronic Hepatitis B Virus Infection on Bone Mineral Density

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Abstract

Background: Chronic hepatitis B virus (HBV) infection is a common health problem that has a worldwide distribution. Apart from the direct effect of the virus on the liver, there are many extrahepatic manifestations among which the probable effect on bone turnover associated with low bone mineral density (BMD). **Objectives:** This study aimed to determine the association between treated and untreated chronic HBV infection with BMD. **Methods:** This is a cross-sectional study which included a total of 48 patients with chronic HBV (28 patients treated with tenofovir-disoproxil-fumarate [TDF] antiviral drug and 20 patients have not yet started treatment). Other age- and sex-matched 30 apparently healthy individuals were recruited to represent the healthy controls. BMD was measured using dual-energy X-ray absorptiometry on the anteroposterior lumbar spine (L1–L4 spine) views, from which T-score was calculated. Liver function tests were also evaluated from serum samples. **Results:** Treated patients showed a lower T-score (−0.48 ± 0.72) than either healthy individuals (1.08 ± 0.84) or untreated patients (0.78 ± 0.51), with highly significant differences. In multivariate regression, only disease duration (adjusted odds ratio [OR] = 9.71, 95% confidence interval [CI] = 4.8–16.68) and TDF treatment (adjusted OR = 6.4, 95% CI = 4.18–97.05) were significantly associated with BMD. **Conclusions:** Prolonged use of TDF in the treatment of HBV infection can significantly reduce BMD. Moreover, BMD can also be inversely affected in long-standing HBV, regardless of treatment regimen.

Keywords: Bone mineral density, chronic hepatitis B virus, tenofovir

Introduction

It was estimated that at least two billion people are infected with hepatitis B virus (HBV), and 240 million individuals suffer from chronic HBV infections.[1] Apart from the direct effect on liver tissue, chronic HBV infection has many extrahepatic manifestations such as autoimmune, hematologic, and dermatologic disorders.[2] Furthermore, HBV was suggested to be a major risk for developing osteopenia, maybe due to increased bone reabsorption and decreased bone formation.[3,4] Such effect of chronic HBV is not always recognized. That is because osteoporosis and low Bone mineral density (BMD) are associated with too many risk factors, such as aging, immobility, hypertension, hyperparathyroidism, use of antihypertensive agents, menopause (in women), diabetes mellitus (DM), corticosteroid usage, low calcium intake, Vitamin D deficiency, and genetic factors.[5]

What further complicated the association between HBV and BMD is that the majority of patients with chronic HBV are on long exposure to antiviral drugs such as tenofovir-disoproxil-fumarate (TDF), which can alter the expression of genes involved in cell signaling, energy, and amino acid metabolism in the osteoclasts and osteoblasts and decrease BMD.[6] Thus, it remains debated whether viral infection itself or long-term exposure to drugs for controlling infection contributes to bone fragility. Therefore, this study aimed to determine the association between treated and untreated chronic HBV infection with BMD.

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Methods

Study population

This is a cross-sectional study which included a total of 48 patients with chronic HBV (age range 26–48 years) who were attending Al-Imamain Al-Kadhimain Medical City, Baghdad, from January 2018 to May 2019. The patients were divided into two groups: (1) those treated with TDF (tenofovir) for 6 months and above (28 patients) and (2) those who were recently considered for treatment with this drug but did not start the treatment (20 patients). Patients with hepatocarcinoma, autoimmune liver disease, celiac disease, chronic kidney disease, or thyroid disorders were excluded from the study. Other age- and sex-matched 30 apparently healthy individuals who were subjected to dual-energy X-ray absorptiometry (DEXA) as an investigation of their general health were also recruited to represent the healthy controls.

Ethical consideration

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patient's verbal approval before sample was taken. This study was approved by the review board of Tropical-Biological Research Unit, College of Science, University of Baghdad.

Patients or healthy subjects who had a history of treatments that could affect bone mass (calcium, bisphosphonates, estrogens, Vitamin D supplements, corticosteroids, and active intravenous drugs) and with a history of hip or lumbar spine fracture were excluded from the study.

Sample collection

Informed consent explaining the dimensions of the study was obtained from each participant before sample collection. About 3 ml of the venous blood was collected in plain tubes from each participant. Sera were separated by centrifugation and kept at −20°C until be used. All serum samples were tested for HBsAg using enzyme-linked immunosorbent assay (Bioprobes, Italy) to confirm the chronic HBV. Patients giving negative results and healthy subjects giving positive results for this test were excluded from the study. Biochemical test was used to detect the liver enzymes and total serum bilirubin to measure the hepatic cellular damage.

Bone mineral density measurements

Patients were offered DEXA as a follow-up for their condition. BMD was measured using DEXA scan (DEXXUM-3-OSTEOSYXS/Korea) by a trained radiology technician on the anteroposterior lumbar spine (L1–L4 spine) views. T-score was calculated as a number of standard deviations (SDs) above or below the mean for a healthy 30-year-old individual of the same sex and ethnicity for the individual studied.[7]

Statistical analysis

All statistical analyses were performed by SPSS version 13 (SPSS, Inc., Chicago, IL, USA). Continuous variables were expressed as mean ± SD and analyzed with analysis of variance. Dichotomous variables were expressed as numbers and frequency and analyzed with Chi-square test. Univariate and multivariate logistic regression were used to find out the predators for low BMD through calculating the odds ratio (OR) and its corresponding 95% confidence interval (CI). Pearson's correlation test was used to explore the possible correlations between T-score and quantitative variables in patients. A $P \leq 0.05$ was considered statistically significant.

Results

Demographic and clinical characteristics of the study population

The mean age of controls, untreated, and treated patients was 34.21 ± 4.46, 37.98 ± 4.76, and 37.98 ± 4.76 years, respectively, with no significant differences between the three groups. Likewise, there were no significant differences between groups in gender distribution, BMI, or smoking habit. However, treated patients had significantly longer duration of HBV infection than untreated patients (68.18 ± 32.7 vs. 27.88 ± 20.93 months). Two comorbidities were reported (DM and hypertension) which had very low incidence that did not differ significantly between the groups [Table 1].

Patients with hepatitis (treated and untreated) had significantly higher serum levels of all parameters associated with liver function parameters than controls. For aspartate transaminase and alanine transaminase (ALT), there were no significant differences between treated and untreated patients. In contrast, treated patients showed significantly higher levels of ALP (121.61 ± 37.14 U/L) than untreated patients (101.4 ± 23.92 U/L), while untreated patients showed significantly higher levels of bilirubin than treated patients (1.78 ± 1.4 mg/dL vs. 1.13 ± 0.68 mg/dL).

T-Score

Mean T-score in healthy individuals was 1.08 ± 0.84 which did not differ significantly from that of untreated patients (0.78 ± 0.51). In contrast, treated patients showed a lower T-score (−0.48 ± 0.72) than either healthy individuals or untreated patients with highly significant differences [Figure 1].

Predictors of low bone mineral density in patients with chronic hepatitis B virus infection

In univariate regression, three factors were significantly associated with low BMD. The vast majority (88.89%) of the patients with T-score = 0 or less had a disease duration of >30 months compared with only 10% of the patients with T-score greater than 0 who had such duration (crude OR = 72.0, 95% CI = 0.8–187). Similarly, about two-third (65%) of the patients with relatively low T-score were received TDF treatment versus 23.33% of the patients with relatively high T-score who received this medication (crude OR = 8.5, 95% CI = 2.25–32.43). In contrast, there was a significant negative association between BMD and ALP. More than half (53.33%) of the patients with a higher T-score showed a serum level of ALP >110 U/L compared with 22.22% of the patients with

lower T-score with such ALP levels (crude OR = 0.25, 95% CI = 0.07–0.94). However, in multivariate regression, only disease duration (adjusted OR = 9.71, 95% CI = 4.8–16.68) and TDF treatment (adjusted OR = 6.4, 95% CI = 4.18–97.05) kept their significant association with BMD [Table 2].

Correlation between T-score and other variable in hepatitis B virus patients

In the untreated group, only the duration of the disease showed a significant negative correlation with T-score (r = −0.361, P = 0.022). In the treated group, T-score showed a significant negative correlation with each of duration (r = −0.469, P = 0.002) and ALT (r = −0.360, P = 0.022) as shown in Table 3.

Discussion

Low BMD is a common health problem that may lead to osteopenia and osteoporosis. The etiologies of low BMD are diverse and include environmental and genetic factors. Some previous reports suggested a significant association between chronic HBV infection and the development of osteopenia.[8,9] Many other studies found that prolonged treatment with some antiviral drugs can increase the risk of osteoporosis.[7,10]

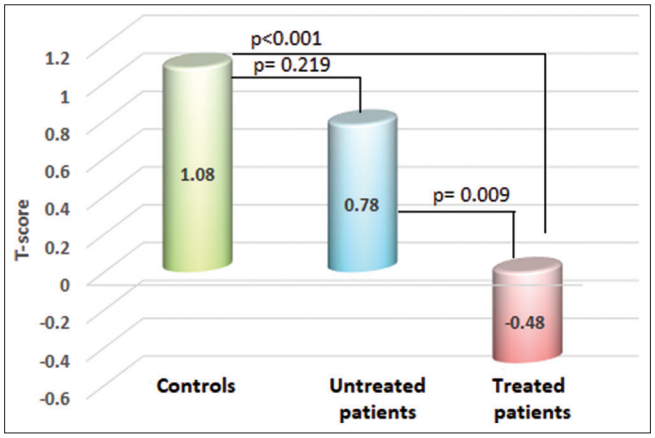

Figure 1: T-score in patients and controls

One important result of the present study was that treatment with TDF can cause a reduction in BMD, compared with normal individuals and untreated cases. Although untreated cases had a trend for decreased BMD compared with healthy individuals, the difference was insignificant.

In accordance with the present study is a British study which included 122 patients treated with TDF as well as 48 patients without treatment. Treated patients showed significantly lower BMD than untreated patients.[7] Furthermore, there was a significant relationship between the mean duration of taking TDF with osteoporosis and osteopenia so that, in patients who had consumed high doses of this drug, osteoporosis and osteopenia were reported more often.[10] In China, Huang *et al*.[9] reported that the prevalence of osteoporosis in either lumbar spine, total hip, or femoral neck was significantly higher in patients with chronic HBV compared with healthy controls. The insignificant difference between untreated and healthy individuals in T-score in the present study can be attributed to the relative small size of the study. Furthermore, the selected site for DEXA and the ethical variation can also affect the results.

Several mechanisms have been proposed to explain the significant effect of TDF on BMD. TDF can directly interfere with the gene expression of osteoblast.[11,12] Alternatively, TDF can affect Vitamin D metabolism and directly drive a state of sustained hyperparathyroidism with an increase in bone turnover. It was reported that parathyroid hormone

Table 1: Demographic characteristics of the study population

Variables	Controls (n=30), n (%)	Untreated patients (n=20), n (%)	Treated patients (n=28), n (%)	P
Age (years)	34.21±4.46	32.92±6.91	37.98±4.76	0.168
Gender				
Male	12 (40)	13 (65)	18 (64.29)	0.105
Female	18 (60)	7 (35)	10 (35.72)	
BMI (kg/m^2)	27.44±6.12	31.72±7.41	32.96±7.14	0.117
Disease duration (months)	-	27.88±20.93	68.18±32.7	<0.001
Smoking				
Never	24 (80)	13 (65)	21 (75)	0.490
Ex/current	6 (20)	7 (35)	7 (25)	
Comorbidities				
DM	2 (6.67)	1 (5)	1 (3.57)	0.868
Hypertension	1 (3.33)	0 (0)	2 (7.14)	
DM + hypertension	0 (0)	1 (5)	1 (3.57)	
AST (IU/L)	28.91±4.32^a	45.44±14.6^b	41.33±14.22^b	0.018
ALT (IU/L)	31.84±7.5^a	53.97±19.2^b	57.53±17.36^b	<0.001
ALP (U/L)	69.54±18.89^a	101.4±23.92^b	121.61±37.14^c	<0.001
TSB (mg/dL)	0.78±0.22^a	1.78±1.4^b	1.13±0.68^c	<0.001

Different small letters indicate significant differences. BMI: Body mass index, DM: Type 2 diabetes mellitus, AST: Aspartate transaminase, AL: Alanine transaminase, ALP: Alkaline phosphatase, TSB: Total serum bilirubin

Table 2: Predictors of low bone mineral density in patients with chronic hepatitis B virus infection

Variables	T-score greater than 0 (n=30), n (%)	T-score=0 or less (n=18), n (%)	Crude OR (95% CI)	Adjusted OR (95% CI)
Age (years)				
≤30	24 (80)	12 (66.67)	1.0	1.0
>30	6 (20)	6 (33.33)	2.0 (0.53-7.54)	2.64 (0.09-74.24)
Gender				
Male	18 (60)	13 (65)	1.0	1.0
Female	12 (40)	5 (35)	0.58 (0.16-2.04)	0.54 (0.04-7.6)
BMI (kg/m^2)				
≤25	5 (16.67)	5 (27.28)	1.0	1.0
>25	25 (83.33)	13 (72.22)	0.52 (0.13-2.13)	0.29 (0.01-6.11)
Duration (months)				
≤30	27 (90)	2 (11.11)	1.0	1.0
>30	3 (10)	16 (88.89)	72.0 (10.8-187)**	9.71 (4.8-16.68)**
Smoking				
Never	21 (70)	13 (65)	1.0	1.0
Ex/current	9 (30)	5 (35)	1.11 (0.31-4.06)	2.48 (0.16-39.55)
AST (IU/L)				
≤40	24 (80)	14 (77.78)	1.0	1.0
>40	6 (20)	4 (22.22)	1.14 (0.27-4.76)	1.74 (0.11-29.0)
ALT (IU/L)				
≤50	7 (23.33)	3 (16.17)	1.0	1.0
>50	23 (76.67)	15 (83.33)	1.52 (0.34-6.82)	3.76 (0.18-76.45)
ALP (U/L)				
≤110	14 (46.67)	14 (77.78)	1.0	1.0
>110	16 (53.33)	4 (22.22)	0.25 (0.07-0.94)*	5.0 (0.41.60.5)
TSB (mg/dL)				
≤1.0	11 (36.67)	2 (11.11)	1.0	1.0
>1.0	19 (63.33)	16 (88.89)	4.63 (0.89-24.04)	3.78 (0.19-75.16)
Treatment (TDF)				
No	23 (76.67)	5 (35)	1.0	1.0
Yes	7 (23.33)	13 (65)	8.5 (2.25-32.43)**	6.4 (4.18-97.05)*

*Significant at 0.05, **Significant at 0.001. BMI: Body mass index, AST: Aspartate transaminase, AL: Alanine transaminase, ALP: Alkaline phosphatase, TSB: Total serum bilirubin, OR: Odds ratio, CI: Confidence interval, TDF: Tenofovir-disoproxil-fumarate

Table 3: Pearson's correlation between T-score and different variables in treated and untreated patients

Variables	Untreated patients		Treated patients	
	R	*P*	*r*	*P*
Age	−0.086	0.599	−0.087	0.596
Duration	−0.361	0.022	−0.469	0.002
BMI	0.128	0.413	0.091	0.574
AST	0.116	0.477	−0.044	0787
ALT	−0.117	0.471	−0.360	0.022
ALP	0.229	0.156	0.180	0.266
TSB	0.213	0.188	0.161	0.120

BMI: Body mass index, AST: Aspartate transaminase, AL: Alanine transaminase, ALP: Alkaline phosphatase, TSB: Total serum bilirubin

levels are elevated early after TDF therapy.[13] Another study showed that high plasma levels of TDF were associated with increased levels of Vitamin D binding receptor, which cause lower free (biologically active) 1,25-hydroxy-vitamin D. This "functional" Vitamin D deficiency may result in secondary hyperparathyroidism.[14]

According to multivariate analysis in the present study, only disease duration and TDF treatment were significantly associated with lower BMD. In partial agreement with these results is the study of Gill *et al*.[7] who demonstrated that TDF therapy was an independent risk factor for low BMD. In other studies, advancing age, lower BMI, and smoking were all associated with significant reductions in BMD on multivariate analysis.[15,16] These variations in the results between different studies may be attributed to variations in the study design, ethnicity, and sample size. Of note, most available literature did not involve the duration of the disease in their analysis.

The significant association of disease duration as an independent risk factor for low BMD regardless of treatment implies that prolonged liver injury will eventually affect bone turnover. In this regard, the release of inflammatory cytokines (e.g., interleukin-1 [IL-1], and IL-6, and tumor necrosis factor-alpha [TNFα]) was mainly accused as the culprit for low BMD. Higher levels of these cytokines can increase receptor activator of nuclear factor kappa-B-ligand to stimulate osteoclastogenesis and bone resorption.[17] Moreover,

TNFα has been shown to have an inhibition effect on osteoblast differentiation, while it promotes the apoptosis of osteoblast.[18] The additive effects of these pro-inflammatory cytokines may reduce bone formation and increase bone resorption, resulting in a decline in BMD with as a consequence of osteoporosis.[9]

The present data confirmed the adverse effect of prolonged TDF on BMD, which may lead to osteopenia or osteoporosis. Moreover, it is obvious that the reduced BMD is a time matter in patients with HBV, regardless of treatment regimen. Therefore, every precaution should be followed by patients (especially older ages) to reduce this risk. Taking enough Vitamin D, regular sun exposure, and having a balanced diet may be considered good measure to reduce this risk.

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Conflicts of interest

There are no conflicts of interest.

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