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Bone Mineral Content in Children with Chronic Hepatitis B: A 12-Year Study

Serdar Göktaş[1*], Nafiye Urganci[2]

[1]SBU. Van Education and Research Hospital, Department of Pediatric Immunology and Allergy, Van, Turkey
[2]SBU. Şişli Hamidiye Etfal Training and Research Hospital, Department of Pediatrics, Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Istanbul, Turkey

ABSTRACT

Chronic Hepatitis B (CHB) is an HBV infection that lasts for more than six months, leading to persistent liver inflammation. This study explored the association between CHB and bone mineral density (BMD). This retrospective study analyzed data from 77 CHB patients applied to the Pediatric Gastroenterology, Hepatology, and Nutrition Unit over a 12-year period. Patient data, including demographics, biochemical markers, liver biopsy findings, and dual-energy X-ray absorptiometry (DXA) Z-scores, underwent comprehensive statistical analysis. The participants' mean age was 9.76 ± 3.87 years, ranging from 3 to 20, with 63.6% of the cohort being male. Histological activity index (HAI) assessments revealed that 32.4% of patients exhibited minimal activity, 50% had mild activity, and 17.6% showed moderate activity; no severe cases were recorded. Fibrosis scores ranged as follows: 18.2% of patients scored 0, 63.6% scored 1, 9.1% scored 2, 5.2% scored 3, and 3.9% scored 4. Low BMD, defined as a DXA Z-score ≤ -2, was detected in 16 patients (20.7%), with no fractures reported. Although female patients had lower DXA Z-scores than males, this difference was not statistically significant (p=0.0531). Moreover, no gender-related differences were observed in low BMD prevalence. HAI and fibrosis scores did not significantly differ between patients with normal and low BMD. However, a positive correlation was observed between HAI and fibrosis scores (r = 0.231, p = 0.044). Expanding DXA screenings in larger CHB patient populations may facilitate the development of more precise guidelines and improve early detection strategies.

Keywords: Chronic hepatitis B, DXA, osteopenia, osteoporosis, Z-score

Introduction

CHB poses a serious global health challenge, defined by liver inflammation that persists for six months or more due to HBV infection. Globally, it is estimated that two billion individuals have been exposed to HBV, with around 240 million currently living with chronic infections (1). Although CHB is mainly linked to liver-related complications, it is increasingly acknowledged for its systemic effects, encompassing various extrahepatic manifestations. HBV is considered a key factor contributing to enhanced bone resorption and diminished bone formation (2). Pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), significantly stimulate osteoclast activity, leading to an accelerated rate of bone resorption (3). Additionally, cytokines like interferon-gamma (IFN-γ) inhibit osteoblast activity, thereby slowing the bone formation process (4). Chronic inflammation generates increased levels of reactive oxygen species, which further impair osteoblast survival and weaken the quality of the bone matrix (5). The connection between HBV and BMD becomes more complex due to prolonged antiviral treatments, such as tenofovir-disoproxil-fumarate (TDF). These therapies can alter gene expression involved in cell signaling, energy metabolism, and amino acid pathways in both osteoclasts and osteoblasts, ultimately reducing BMD (6). Bone health disorders associated with CHB pose major public health concerns, driven by their increasing socioeconomic burden, high rates of morbidity and mortality, and growing prevalence. This study aims to explore the association between low bone mineral density and CHB, while contributing to improved strategies for managing bone health in CHB patients.

Materials and Methods

Sample size estimation was conducted using G*Power software to ensure sufficient statistical power. Two statistical approaches were used: For correlation analysis, assuming a moderate effect

***Corresponding Author:** Serdar Göktaş, SBU Van Education and Research Hospital, Department of Pediatric Immunology and Allergy, Van, Turkey
E-mail: drsrdr33@gmail.com, Phone: +90 505 496 9028
ORCID ID: Serdar Göktaş: 0000-0002-5738-4979, **Nafiye Urganci:** 0000-0003-4854-507X
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size (r = 0.50), a statistical power of 0.80, and a Type I error rate (α) of 0.05, the minimum required sample size was 29, based on the appropriate formula. For the independent two-group t-test, assuming a large effect size (r = 0.80), a statistical power of 0.80, and a Type I error rate of 0.05, the required sample size was 52, using the corresponding formula. The study included children aged 3–20 years diagnosed with chronic hepatitis referred to the Pediatric Gastroenterology, Hepatology, and Nutrition Department. Demographic data, including age and gender, were recorded [mean age: 9.76 ± 3.87 years; 63.6% male (n = 49), 36.4% female (n = 28)].

Bone mineral density (BMD) was assessed using DXA, with Z-scores adjusted for age, height, and gender. A Z-score ≤ -2 was defined as low BMD, while an osteoporosis diagnosis required evidence of a fracture. Based on these classifications, patients were divided into two groups: normal BMD (DXA Z-score > -2) and low BMD (DXA Z-score ≤ -2). The results were evaluated in comparison with the 2006 reference values for normal bone mineral density in Turkish children (7). Histopathological parameters, including HAI and fibrosis scores, were obtained from liver biopsies. Patients were categorized based on disease activity levels (minimal, mild, moderate, severe) and fibrosis stages (0–4) using standard scoring criteria. To minimize the confounding effects of antiviral therapies, only data at diagnosis were analyzed.

Statistical Analysis: Data analysis was conducted using SPSS (ver. 18.0). Descriptive statistics were reported as means, standard deviations, and minimum-maximum values for continuous variables, and as counts and percentages for categorical variables. Shapiro-Wilk test was used to assess normality. Mann-Whitney U test was applied to two-group comparisons of non-normally distributed continuous variables. Spearman's correlation test was used to evaluate relationships between continuous variables. Pearson's chi-square test analyzed categorical data. Fisher's exact test and exact chi-square test were performed when applicable. Statistical significance was set at $p < 0.05$ (95% confidence level).

Results

A group of 77 children diagnosed with CHB, aged between 3 and 20 years (mean age: 9.76 ± 3.87 years), participated in this study. The cohort was composed of 63.6% males (n = 49) and 36.4% females (n = 28) (Table 1).

There were no statistically significant differences in the biochemical parameter analyses between their respective categories of calcium (Ca) (p=0.369), phosphorus (P) (p=1.000), magnesium (Mg) (p=1.000), alanine aminotransferase (ALT) (p=0.842), alkaline phosphatase (ALP) (p=0.928), parathyroid hormone (PTH) (p=0.099), and 25(OH) Vitamin D (p=0.859) with respect to DXA Z-scores; however, a statistically significant difference was observed for aspartate aminotransferase (AST) (p<0.001) (Table 2).

Based on HAI stratification, 37.4% of the children displayed minimal disease activity, 50.0% had mild activity, and 17.6% were classified as having moderate activity. Importantly, no cases of severe disease activity were documented (Fig. 1A). The fibrosis score distribution was as follows: 18.2% had a score of 0, 63.6% scored 1, 9.1% scored 2, 5.2% scored 3, and 3.9% scored 4 (Fig. 1B).

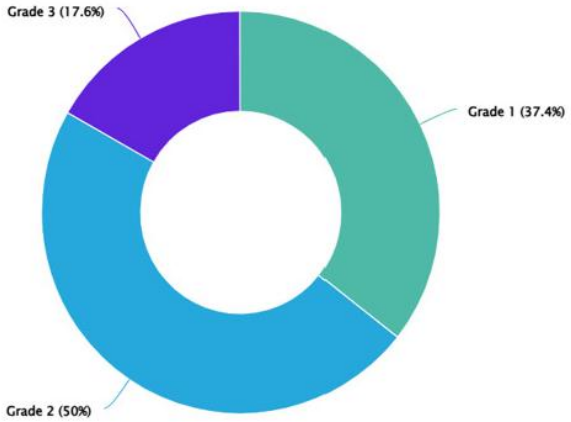
Low BMD (DXA Z-score ≤ -2) was observed in 20.7% of participants (n = 16) (Table 3). Notably, no fractures were reported in the study population. Gender-based analysis showed significantly lower DXA Z-scores in females compared to males (p = 0.0065). Nevertheless, there were no statistically significant differences between males and females in terms of DXA Z-score (p=0.053), HAI score (p=0.368), and fibrosis score (p=0.909) in the Low BMD group. Similarly, in the Normal BMD group, no statistically significant differences were found for DXA Z-score (p=0.437), HAI score (p=0.984), and fibrosis score (p=0.611).

Additionally, no significant variation in total HAI (p=0.636) or fibrosis scores (p=0.534) was identified between children with normal BMD and those with low BMD. A weak but statistically significant positive correlation was noted between HAI scores and fibrosis scores ($\rho = 0.231$, $p = 0.044$) (Table 4).

Discussion

CHB infection is primarily known for its hepatic complications; however, it is increasingly recognized as a systemic disease with far-reaching effects. In this study, we explored the association between CHB and low BMD. Studies evaluating BMD have also been conducted on other chronic inflammatory conditions like CHB (8-10). Our findings revealed that low BMD prevalence in CHB patients is significantly higher than in healthy populations.

The mechanisms underlying CHB's impact on bone health have been described in the literature. Chronic inflammation enhances osteoclastic activity,



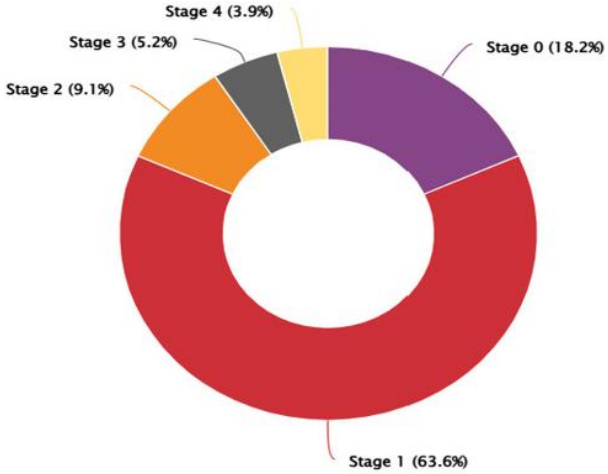


Fig. 1A. HAI-Necroinflammatory Activity Grades (Grade 1: Minimal activity, Grade 2:Mild acitivity; Grade3:Moderate activity).
Fig. 1B. Knodell Fibrosis Score (Stage 0: No fibrosis, Stage 1: Portal fibrosis without septa, Stage 2: Portal fibrosis with rare septa, Stage 3: Septal fibrosis with many bridging septa, Stage 4: Cirrhosis (extensive fibrosis with nodular architecture)

Table 1. Demographic Findings

		n (%)
Gender	Male	49 (63.6%)
	Female	28 (36.4%)
	Min-Max	Mean ± SD
Age	3-20	9.76±3.87
Weight	12-80	32.06± 14.52
Height	96-180	131.58±19.74
BMI	12.2-28.7	17.57± 3.01

SD: Standard deviation; Min: Minimum; Max: Maximum

increasing bone resorption. This process is mediated through cytokines, particularly pro-inflammatory cytokines such as IL-6 and TNF-α, which disrupt bone homeostasis (11). This may partly explain the higher prevalence of low BMD observed in CHB patients. Secondary malabsorption, nutritional deficiencies, vitamin K deficiency, cholestasis, and hypogonadism associated with chronic inflammatory conditions also contribute to impaired bone health.

Additionally, antiviral agents such as TDF adversely affect bone health by inhibiting osteoblastic activity. Long-term use of nucleotide/nucleoside analogs like TDF can cause mitochondrial toxicity, suppressing osteoblast function (12). Moreover, TDF-induced renal tubular dysfunction results in Ca and phosphate loss, further impairing bone health. Although our study took into account the patients' data at the time of diagnosis to eliminate TDF effects, the literature emphasizes the significant effects of such antiviral treatments on BMD (13).

Gender differences also emerged as a significant factor. Although female patients had lower DXA-Z scores overall, this difference was not statistically significant (p=0.0531). This aligns with the regulatory role of estrogen in bone metabolism, where estrogen deficiency increases osteoclastic activity, resulting in reduced bone density (14). Consequently, bone health in female CHB patients warrants special attention.

Interestingly, no association was observed between HAI or fibrosis scores and low BMD, suggesting that CHB effects on bone health may involve more complex mechanisms. However, the positive correlation between HAI and fibrosis scores ($r = 0.231$, $p = 0.044$) indicates that biological processes linked to liver damage progression could potentially influence bone health. Future studies with larger cohorts may provide further insights into the impact of advanced fibrosis on BMD.

Most biochemical parameters did not show significant differences according to DXA Z-scores; however, AST levels were significantly higher in the low BMD

Table 2: Evaluation of Biochemical Parameters Based on DXA Z-Scores

		Low BMD (DXA z score ≤ -2)	Normal BMD (DXA Z score > -2)	p
		n (%[1])	n (%[1])	
Ca	Low	3 (12.5)	3 (5.7)	0.369[2]
	Normal	21 (87.5)	50 (94.3)	
P	Low	1 (4.2)	4 (7.5)	1.000[2]
	Normal	23 (95.8)	49 (92.5)	
Mg	Low	---	2 (3.8)	1.000[2]
	Normal	24 (100.0)	51 (96.2)	
ALT	Normal	13 (54.2)	30 (56.6)	0.842[3]
	High	11 (45.8)	23 (43.4)	
AST	Normal	3 (12.5)	28 (52.8)	<0.001[3]
	High	21 (87.5)	25 (47.2)	
ALP	Low	5 (20.8)	10 (18.9)	0.928[4]
	Normal	16 (66.7)	38 (71.7)	
	High	3 (12.5)	5 (9.4)	
PTH	Normal	19 (79.2)	50 (94.3)	0.099[2]
	High	5 (20.8)	3 (5.7)	
25(OH) vitD	Low	5 (20.8)	12 (22.6)	0.859[3]
	Normal	19 (79.2)	41 (77.4)	

[1]Column percentage; [2]Fisher's Exact test; [3]Pearson chi-square test; [4]Exact chi-square test

Table 3: Distribution of Patients With Low and Normal BMD According to HAI and Fibrosis Scores

	Low BMD (DXA Z score ≤ -2)						Normal BMD (DXA Z score > -2)					
	DXA Z score		HAI score		Fibrosis score		DXA Z score		HAI score		Fibrosis score	
Gender	n mean± SD	p	n mean± SD	p	n mean± SD	p	n mean±SD	p	n mean± SD	p	n mean ±SD	p
Male	8 -2.47±0.69	0.0531	8 6.28±3.90	0.3681	8 1±0.81	0.9091	41 1.073±0.78	0.4371	41 5.39±2.83	0.9841	41 1.023±0.56	0.6111
Female	8 -3.91±1.43		8 4.11±3.25		8 0.88±0.33		20 1.4±1.27		20 5.4±3.33		20 1.21±1.45	

SD: Standard deviation; 1Mann-Whitney U test

No significant differences were observed in DXA Z-scores, HAI scores, and fibrosis scores between males and females in both the low (DXA Z-score ≤ -2) and normal BMD (DXA Z-score > -2) groups.

group than the normal BMD group (p<0.001). Previous studies report a lack of a correlation between biochemical parameters and BMD. However, our study identified a significant association between AST levels and low BMD. This finding suggests a potential relationship between AST and bone mineral density, underscoring the need for further comprehensive research to explore this association in greater detail (15-17).

Normal levels of Ca, P, Mg, and 25(OH) vitamin D in our cohort suggest that inflammatory processes may be the primary contributors to low BMD. However, monitoring PTH and ALP levels may provide a more dynamic evaluation of bone metabolism. While ALP

Table 4: Comparison of HAI Score and Fibrosis Between Low and Normal BMD Groups and Their Correlation

		Low BMD (DXA Z score ≤ -2)	Normal BMD (DXA Z score > -2)	p
HAI score	mean±SD	5.06±3.60	5.32±2.98	0.6361
Fibrosis	mean±SD	0.93±0.57	1.18±0.97	0.5341
Correlation (HAI score vs Fibrosis, overall)		ρ=0.231		0.044

SD: Standard deviation

1Mann-Whitney U test; 2Spearman correlation test

No significant differences were found in HAI score and fibrosis between the BMD groups ($p > 0.05$), but a weak positive correlation between HAI score and fibrosis was significant ($p = 0.044$).

has been linked to low BMD in some studies, no significant difference was observed in our study. This discrepancy could be further explored in studies with larger sample sizes. Additionally, although vitamin D deficiency was not prominent in our cohort, it may become more evident in patients with advanced fibrosis.

In conclusion, the high prevalence of low BMD in CHB patients underscores the need for prioritizing bone health in this population. Future studies should aim to better understand the impact of CHB and antiviral therapies on bone metabolism and develop strategies to mitigate these effects.

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Conflict of Interest: The authors have no conflict of interest.

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