

Evaluation of Bone Mineral Density and Bone Turnover Markers in Patients with non-Cirrhotic Chronic Hepatitis B

Sirotik Olmayan Kronik Hepatit B'li Hastalarda Kemik Mineral Yoğunluğu ve Kemik Döngüsü Göstergelerinin Değerlendirilmesi

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ABSTRACT Objective: To evaluate the bone mineral density (BMD) and the biochemical markers of bone turnover in non-cirrhotic chronic hepatitis B patients. **Material and Methods:** We compared 18-65 years old non-cirrhotic chronic hepatitis B patients and healthy controls for BMD and biochemical markers of bone turnover. BMD was measured at the lumbar spine and the left hip using DXA. **Results:** There were 31 hepatitis B patients (19 males, 12 females) and 72 healthy controls (56 males, 16 females) in the study. Their mean age was 37.4 ± 10.0 years in the study group and 38.5 ± 7.1 years in the controls. There were no differences between the two groups for the mean BMD levels and T scores. However, left hip BMD and T scores were significantly lower in male patients with chronic hepatitis B infection when compared to males in the control group. Serum calcium, phosphate, osteocalcin, intact parathyroid hormone, deoxypyridinoline and 25-hydroxycholecalciferol levels did not differ between the groups. **Conclusion:** In our study, left hip BMD and T scores were found to be significantly lower in the male patients with chronic hepatitis B infection when compared to males in the control group, however a significant difference was not detected when all cases were compared with the control group. Therefore, we suppose that further studies on a larger population are needed in this issue.

Key Words: Hepatitis B virus; chronic hepatitis B; bone density

ÖZET Amaç: Sirotik olmayan kronik hepatit B'li hastalarda kemik mineral yoğunluğu (KMY) ve kemik döngüsü biyokimyasal parametrelerini değerlendirmektir. **Gereç ve Yöntemler:** Yaşları 18-65 arasında olan, sirotik olmayan kronik hepatit B'li hastaların ve sağlıklı olguların KMY ve kemik döngüsü biyokimyasal parametrelerini karşılaştırdık. KMY ölçümü, DXA yöntemi ile lomber omurga ve sol kalça bölgesinden yapıldı. **Bulgular:** Çalışmaya 31 hepatit B hastası (19 erkek, 12 kadın) ve 72 sağlıklı kişi (56 erkek, 16 kadın) alınmıştır. Yaş ortalaması kronik hepatit B'li grupta $37,4 \pm 10$ yıl, sağlıklı kontrol grubunda $38,5 \pm 7,1$ yıl olarak hesaplandı. Ortalama KMY değerleri ve T skorları açısından iki grup arasında anlamlı fark saptanmadı. Ancak, sol kalça KMY ve T skorları kronik hepatit B'li erkek hastalarda kontrol grubundaki erkeklere göre anlamlı ölçüde daha düşük bulunmuştur. Serum kalsiyum, fosfat, osteokalsin, aktif parathormon, deokspiridinolin ve 25-hidroksikalsiferol seviyeleri yönünden iki grup arasında anlamlı fark saptanmamıştır. **Sonuç:** Çalışmamızda kronik hepatit B'li erkek hastaların sol kalça KMY ve T skorları sağlıklı erkeklere göre anlamlı düzeyde düşük bulunmuştur ancak tüm vakalar kontrol grubu ile karşılaştırdığımızda anlamlı bir fark saptanmamıştır. Bu nedenle bu konuda daha geniş vaka grupları ile yapılacak ileri çalışmalara gerek olduğunu düşünmekteyiz.

Anahtar Kelimeler: Hepatit B virüsü; kronik hepatit B; kemik yoğunluğu

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AIteration in mineral amount of bone is a common complication of chronic liver disease.^{1,2} Hepatic osteodystrophy, described by Heaf, is a general definition that describes abnormalities in mineral density

and bone metabolism in individuals with chronic liver disease.³ Prevalence of osteoporosis has been reported between 9% and 100% among the patients with chronic liver diseases in which cholestatic disorders and alcoholic liver disease have the highest rates.⁴⁻⁸ Imbalance between osteoblast-mediated bone formation and osteoclast-induced bone resorption is considered as the main mechanism of osteoporosis in progressive liver disease.^{1,2} The elements in potential mechanism of bone loss in progressive liver disease are hypothesized as hypogonadism (estrogen and testosterone deficiency), reduced hepatic hydroxylation of vitamin D, reduced osteoblastic activity associated with impaired hepatic production of insulin-like growth factor-1, increased osteoclastic activity due to impaired hepatic production of osteoprotegerin and increased level of proinflammatory cytokines.^{1,2} Osteodystrophy in patients with liver cirrhosis secondary to viral hepatitis has been well defined; however, there is little information about occurrence of bone disease in non-cirrhotic patients with chronic viral hepatitis, especially in asymptomatic hepatitis B virus (HBV) carriers.⁹⁻¹³ Therefore, the aim of this study was to evaluate the bone mineral density (BMD) and the biochemical markers relevant the bone turnover in non-cirrhotic patients with chronic viral hepatitis B.

MATERIAL AND METHODS

This prospective study was conducted according to the "Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects" in Zonguldak University Hospital. The study protocol was approved by ethical committee of our hospital and a written informed consent was obtained from each subject. We compared 18-65 years old non-cirrhotic chronic hepatitis B patients and age-gender adjusted healthy controls for BMD and biochemical markers of bone turnover. The control group consisted of the subjects who were randomly selected from an otherwise healthy population aged between 18-65 years and had a negative serology for HIV, HCV and HBsAg.

Chronic hepatitis B was defined as being positive for HBsAg for more than six months. The case group was consisted of the chronic hepatitis B pa-

tients who were evaluated with detailed clinical examination, abdominal ultrasonography (USG), and routine laboratory measurements with 6-12 months intervals in our institution. An inactive HBsAg carrier was defined as a patient who had been positive for HBsAg for more than six months, had persistently normal ALT levels on repeated measurements during a follow-up period of at least one year and a negative or a low level ($<10^4$ copy/ml) of HBV-DNA.^{14,15} Liver biopsy was not routinely implemented for the patients with chronic hepatitis B, however, patients who had elevated ALT levels (1.5 or more times higher than the upper limit of normal value) or high level of HBV-DNA ($\geq 10^4$ copy/ml) were evaluated with a liver biopsy. Chronic hepatitis B patients who were diagnosed as cirrhosis with liver biopsy or who had any finding (clinical, laboratory or imaging) compatible with liver cirrhosis were not included in this study.

Exclusion criteria: The patients in the case group who were concurrently positive for HIV, HCV or HDV were excluded. The participants of the study who had a positive history of the following systemic diseases and disorders were excluded either from the case group or the control group: diabetes mellitus, chronic renal disease, thyroid diseases, parathyroid diseases, current or previous malignancy, connective tissue diseases, current or previous alcohol abuse, previously diagnosed osteoporosis, being on medications for osteoporosis, hormone therapy or any medication affecting bone metabolism such as steroids, anti-epileptics, heparin etc. Postmenopausal females were also excluded in both groups.

BONE MINERAL DENSITY MEASUREMENTS

Bone mineral density was measured at the lumbar spine (L1-4) and the left hip (total hip) using DXA (Hologic QDR4500W, Hologic, Inc Bedford, USA). The results were measured on g/cm² and the number of standard deviation (SD) based on a comparison with peak bone mass (T score). The precision error for the lumbar spine, hip and forearm were 1% for all regions. For quantification of bone mass loss, World Health Organization (WHO) criteria which were defined for postmenopausal women was used.¹⁶ Normal BMD is defined as BMD < -1

SD below mean normal young adult BMD, osteopenia is defined as BMD between -1 and -2.5 SD below mean normal young adult BMD and osteoporosis is defined as BMD > -2.5 SD below mean normal young adult BMD.

BIOCHEMICAL MEASUREMENTS

Blood was drawn in the morning after an overnight fasting. Serum was analyzed for glucose, calcium (Ca), phosphate (P), alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST) and urea, and urine and serum creatinine spectrophotometric analysis was performed with Cobas Integra 800 (Roche Diagnostics, Mannheim, Germany). Free T3, free T4, TSH and intact parathyroid hormone (iPTH) levels were measured using chemiluminescent immunometric assay with Immulite 2000 (BioDPC, LA, US). Osteocalcin was measured using chemiluminescent immunometric assay with Immulite One (BioDPC, LA, US). Deoxypyridinoline (DPD) was measured using chemiluminescent enzyme labeled assay with Immulite One (BioDPC, LA, US). DPD and urine creatinine (UCr) was measured using the second morning urine or from 24-h collection specimens using standardized commercial assays. To correct for variations in urinary flow, DPD results should be normalized according to the urinary creatinine concentration, and expressed as nanomoles DPD, per liter divided by milimoles creatinine per liter (nM DPD/ mM creatinine)(Urinary excretion was expressed as the ratio of DPD to UCr.) 25-hydroxycholecalciferol [25(OH) D3] (Immundiagnostik AG, Bensheim and Biomedica, Wien) was measured by competitive ELISA assays with LP-400 ELISA (Pasteur Diagnostics,FR) system. All samples were stored at -70°C until tested.

STATISTICAL ANALYSIS

Descriptive values were calculated as mean±standard deviation (SD), count and percent. Normality test were done for continuous variables by using Kolmogorov-Smirnov test. After that, student t-test or MannWhitney U test for two independent two groups and Pearson Chi-square test were used first because unadjusted p values were obtained. After,

Covariance analysis was used for differences between groups, sex and its interactions on markers of bone metabolism for adjusting p values were obtained. In this analysis age, body mass index (BMI), smoking, alcohol intake, daily calciums intake, ALT, AST, ALP, glucose, urea, creatine, Ca, P, TSH, free T3 and free T4 were accepted covariates. Type I error probability was accepted as 5%.

RESULTS

There were 31 (19 males, 12 females) patients with chronic hepatitis B in the case group and 72 (56 males, 16 females) healthy individuals in the control group. All females in both groups were premenopausal. In case group, 28 patients were HBeAg negative and anti-HBeAg positive and the remaining three were HBeAg positive and anti-HBeAg negative. Among hepatitis B patients, 28 were inactive HBsAg carriers and the remaining three had mild hepatitis according to Knodell histological activity score in liver biopsy. The mean age was 37.4±10.0 years in case group and 38.5±7.1 years in the controls. There were statistically significant differences between the mean values of (cases and controls respectively) AST (27.2±13.4 and 20.0±5.0 U/L, p<0.001), urea (23.7±6.4 and 28.2±7.4 mg/dL, p=0.004), free T3 (3.50±0.4 and 2.0±0.3 pg/mL, p<0.001), TSH (1.23±0.9 and 1.65±1.2 µIU/mL, p=0.027) and free T4 (1.19±0.2 and 0.89±0.2 ng/dL, p<0.001). However, TSH, free T3 and free T4 levels were all in normal range in both groups. In hepatitis B group, daily calcium intake was insufficient for 83.9% of the cases whereas insufficient daily calcium intake was evident in 26.4% of the controls and the difference was statistically significant (p<0.001). Demographic features and the baseline values of serum biochemical parameters of the hepatitis B patents and the controls are shown in Table 1.

COMPARISONS OF BIOCHEMICAL MARKERS OF BONE TURNOVER

The mean serum iPTH levels in the cases and the controls were 48.9±16.8 and 40.0±14.7, respectively. The difference was statistically significant with student-T test (p=0.010); however, it was not with covariance analysis (p=0.918). There was a

TABLE 1: Demographic characteristics and the mean values of serum biochemical parameters of the hepatitis B patients and the controls.

Variable	Chronic Hepatitis B patients (n=31)	Control group (n=72)	p value
	Mean±SD, (min-max) or (%)	Mean±SD, (min-max) or (%)	
Age	37.4±10.0 (20-54)	38.5±7.1 (26-58)	0.524
Sex			0.086
Male	19 (61.3%)	56 (77.8%)	
Female	12 (38.7%)	16 (22.2%)	
BMI (kg/m ²)	24.89±3.5 (13.60-30.50)	26.78±4.1 (19-37.5)	0.027
Smoking	11/31 (35.5%)	17/72(23.6%)	0.218
Alcohol use	5/31(16.1%)	3/72(4.2%)	0.038
Daily Ca intake			<0.001
Insufficient	26 (83.9%)	19 (26.4%)	
Medium	5 (16.1)	36 (50.0%)	
Sufficient	0 (0%)	2 (2.8%)	
ALT	33.3±27.1 (8-124)	25.6±11.8 (9-69)	0.386
AST	27.2±13.4 (14-77)	21.0±5.0 (9-34)	<0.001
ALP	68.8±23.6 (34-145)	73.9±27.9 (32-249)	0.377
Glucose	89.6±13.8 (70-153)	93.9±11.4 (73 -155)	0.101
Urea	23.7±6.4 (10-40)	28.2±7.4 (4-48)	0.004
Creatinine	0.7±0.2 (0.5-1.1)	0.8±0.1 (0.3-1.2)	0.57
Calcium	9.5±0.5 (7.7 - 10.2)	9.6±0.5 (7.8-10.6)	0.915
Phosphate	3.3±0.5 (2.5-4.5)	3.3±0.6 (1.9-5.0)	0.648
TSH	1.23±0.9 (0.36-4.50)	1.65±1.2 (0.36-5.00)	0.027
Free T3	3.50±0.4 (2.20-4.20)	2.00±0.3 (1.56-3.36)	<0.001
Free T4	1.19±0.2 (0.8-1.60)	0.89±0.2 (0.8-1.95)	<0.001

ALT (normal range < 42 U/L), AST (normal range < 37 U/L, ALP (normal range for females 35-108 IU/L, for males 40-129 IU/L), glucose (normal range 70-110 mg/dL), urea (normal range 10-50 mg/dL), creatinine (normal range(0.5-1.2 mg/dL), calcium (normal range 8.4-10.2 mg/dL), phosphate (normal range 2.5-4.5 mg/dL), TSH (normal range 0.4-5.0 µIU/mL), free T3 (normal range 1.6-4.7 pg/mL), free T4 (normal range 0.8-1.9 ng/dL). SD: Standard deviation; BMI: Body mass index.

statistically significant difference in the mean DPD levels between the cases and the controls (7.2±2.6 and 6.1±1.8, respectively) with student-T test (p=0.020). However, the difference was not statistically significant with covariance analysis (p=0.557). The mean values of İPTH and 25(OH)D3 were not statistically significant different between the groups (Table 2).

COMPARISONS OF BMD MEASUREMENTS

The mean values of lumbar spine BMD in chronic hepatitis B patients and in controls were similar (1.017±0.1 and 1.049±0.1, respectively). No statistically significant differences were detected between the groups in comparisons of lumbar spine T score, total femur BMD or total femur T score (Table 2).

In comparisons for each gender separately (male versus male and female versus female), there was no significant difference between the females of the two groups; however in males, total femur BMD (0.990 versus 1.176 p=0.007) and total femur T score (-0.29 versus 0.92, p=0.009) were higher in male controls. The differences were statistically significant with covariance analysis (Table 3).

DISCUSSION

The mechanism of osteoporosis associated with chronic liver disease is not completely understood, however as in senile osteoporosis, imbalance between osteoblast-mediated bone formation and osteoclast-induced bone resorption is believed to be the main reason of osteoporosis in chronic liver dis-

TABLE 2: Comparisons of bone metabolism markers and BMD values between the hepatitis B patients and the controls.

Variable*	Chronic Hepatitis B patients	Control group	Unadjusted p value according to student-T test	Adjustable p value according to Covariance analysis
	(n=31) mean±SD min-max)	(n=72) mean±SD (min-max)		
Osteocalcine	8.7±5.5 (1.2-21.3)	8.0±4.5 (1.0-25.8)	0.541	0.616
iPTH	48.9±16.8 (19.9-84.0)	40.0±14.7 (17.0-77.5)	0.010	0.918
DPD	7.2±2.6 (2.8-15.0)	6.1±1.8 (3.2-13.4)	0.020	0.557
25(OH)D3	64.9±61.0 (2.7-227.0)	41.9±49.9 (1.6-202.0)	0.080	0.216
Lumbar spine BMD (g/cm ²)	1.017±0.1 (0.751-1.260)	1.049±0.1 (0.847-1.443)	0.182	0.141
Lumbar spine T score	-0.50±1.0 (-3.1-1.7)	-0.27±1.0 (-2.2-3.2)	0.281	0.174
Total femur BMD (g/cm ²)	0.969±0.1 (0.718-1.326)	1.037±0.1(0.705-1.358)	0.030	0.344
Total femur T score	-0.20±0.9 (-1.9-1.9)	0.14±0.9 (-1.9-2.1)	0.076	0.491

Osteocalcine (normal range 3.1-13.7 ng/mL), iPTH (normal range 10-87 pg/mL), DPD (normal range for male 2.3-5.4 for female 3.0-7.4 nM DPD/ mM creatinine), [25(OH)D3] (normal range 25-125 nmol/L).

* In this analysis age, body mass index, smoking, alcohol intake, daily Ca intake, ALT, AST, ALP, glucose, urea, creatine, Ca, P, TSH, free T3 and free T4 were accepted covariates. SD: Standard deviation.

TABLE 3: Comparisons of bone metabolism markers and BMD values between hepatitis B patients and controls in each sex separately. The table includes the “corrected” mean values of the participants which calculated according to covariance analysis.

Variable*	Male			Female		
	Chronic Hepatitis B		Adjustable p value according to Covariance analysis	Chronic Hepatitis B		Adjustable p value according to Covariance analysis
	patients (n=19) mean±SD	Control group (n=56) mean±SD		patients (n=12) mean±SD	Control group (n=16) mean±SD	
Osteocalcine	6.6±2.7	9.8±1.8	0.286	8.4±6.1	11.0±7.5	0.814
iPTH	35.7±9.4	36.3±6.4	0.955	16.5±25.9	68.5±20.9	0.263
DPD	6.4±1.2	6.9±0.9	0.906	3.09±3.5	13.6±4.6	0.281
25(OH)D3	97.1±44.6	52.7±18.6	0.335	100.7±95.4	79.3±110.7	0.383
Lumbar spine BMD (g/cm ²)	1.040±0.06	1.182±0.04	0.05	1.043±0.2	0.864±0.2	0.578
Lumbar spine T score	-0.44±0.6	0.81±0.4	0.05	0.31±1.3	-2.57±1.5	0.298
Total femur BMD (g/cm ²)	0.990±0.06	1.176±0.04	0.007	1.013±0.2	0.706±0.2	0.374
Total femur T score	-0.29±0.4	0.92±0.3	0.009	0.62±1.3	-1.89±1.6	0.369

* In this analysis age, body mass index, smoking, alcohol intake, daily ca intake, ALT, AST, ALP, glucose, urea, creatine, Ca, P, TSH, free T3 and free T4 were accepted covariates. SD: Standard deviation.

ease.^{1,2} Multiple mechanisms that cause bone loss were described in different types of liver diseases. For instance, elevated levels of interleukin-17 which induces osteoclastic activity is considered to be responsible of bone loss in alcoholic liver disease or oncofetal fibronectin produced by activated stellate cells are suggested to suppress osteoblasts and bone formation in patients with primary biliary cirrhosis. The potential mechanism of bone loss in non-cirrhotic chronic hepatitis B is not clearly known. However, some studies have sug-

gested that elevated levels of serum cytokines associated with viral hepatitis, such as tumor necrosis factor-alpha, interleukin-1 and interleukin-6 may inhibit osteoblast-mediated bone formation or promote osteoclast-induced bone resorption which finally lead to reduced BMD.^{1,2}

Osteodystrophy in patients with liver cirrhosis secondary to viral hepatitis has been well defined, however there is limited number of the published studies that investigate bone metabolism in patients with non-cirrhotic viral hepatitis (especially in

asymptomatic HBV carriers) in Pubmed.⁹⁻¹³ In a study performed by Tsuneoka et al. in 1993-1994, 20 non-cirrhotic subjects with chronic viral hepatitis due to HBV (n=8) and HCV (n=12) were compared for bone metabolism with cirrhotic patients and also with age-sex matched healthy volunteers.⁹ BMD was significantly decreased in patients with liver cirrhosis and, to a lesser extent, in patients with chronic hepatitis, compared with healthy subjects. In another study, Schiefke et al. investigated BMD and bone turnover markers in biopsy-proven non-cirrhotic 43 patients (30 with HCV and 13 with HBV) with viral hepatitis.¹² They classified the patients according to Knodell histological staging system and found that the BMD was significantly lower and iPTH level was significantly elevated in more advanced stages of liver disease. The study also demonstrated that BMD and T-scores were lower in patients with chronic hepatitis C than in patients with chronic hepatitis B, but the difference was not statistically significant. In another study published in 2006, Yenice et al. assessed bone metabolism and mineral density in 105 non-cirrhotic chronic viral hepatitis patients (60 HBV and 45 HCV) who were diagnosed with liver biopsy which showed moderate and severe hepatitis activation with elevated serum ALT levels (1.5-2 times higher than normal limits).¹³ They found that the mean T score (BMD measurements were performed at medial phalanges) was significantly higher and the mean PTH level was significantly lower in male patients between 20-40 years of age with chronic hepatitis B who were compared with age-sex matched healthy controls. The mean P level in male patients between 40-65 years old with chronic hepatitis B and the mean DPD level in postmenopausal female patients with chronic hepatitis B were significantly lower when compared with age-sex matched healthy controls. The characteristics of the patients, the etiologic agents of chronic hepatitis (HBV or HCV), the stage of the liver disease (mild to severe) and the model of statistical analysis had some heterogeneity in the above studies and in the present study. Our study was performed only on the patients with hepatitis B, and 28 of them were inactive HBV carriers whereas three of them had mild hepatitis. Therefore, com-

paring the results of the previous studies and the current study may have some limitations. However, in contrast to Yenice et al.'s study, the mean value of total femur BMD and the mean value of total femur T score were significantly lower in males with inactive hepatitis B in the current study.¹³ The mean value of iPTH levels was significantly elevated in the chronic hepatitis (B and C) patients with more advanced stages of liver disease in Schiefke et al.'s report.¹² Similarly, iPTH and DPD levels were significantly higher in patients with inactive hepatitis B in our study. However, the difference was not statistically significant when the statistical analysis was performed with covariance model in our study.

In a recently published study, Lo Re III et al. compared 625 HIV subjects co-infected with hepatitis B or hepatitis C with 612 age-sex adjusted HIV mono-infected individuals for BMD scores.¹⁷ The mean BMD Z scores at the lumbar spine and femoral neck were lower among hepatitis-co-infected women compared to HIV-mono-infected women either without adjustment or after adjustment in statistical method. In contrast to women, no differences for BMD Z scores (without adjustment or after adjustment) were observed among mono-infected and co-infected men. In our study, however not statistically significant, the mean T score (lumbar spine or total femur) in the patients with hepatitis B was lower than in health individuals. When compared each sex separately, the mean adjusted femur T score in the male patients with hepatitis B was lower than in the healthy males (p=0.009) while a significant difference for the mean T score was not observed between the healthy women and the women with hepatitis B.

Chronic inflammatory conditions may stimulate activation of some cytokines such as TNF-alpha, IL-1, IL-3, IL-6, IL-7, IL-11, IL-13, IL17 etc.¹² In many studies serum cytokine levels in the patients with HBV infection (asymptomatic HBV carrier or chronic hepatitis B or acute hepatitis B) were detected to be higher than in healthy donors.^{18,19} In our study, the mean BMD and T scores were found to be lower in the male patients with hepatitis B than in the healthy males. Serum cytokine levels in the subjects were not investi-

gated, however the possible mechanism of low BMD and T scores in the male patients with hepatitis B in our study may be associated with elevated cytokine levels due to chronic inflammation in liver.

There are some limitations in our study. The number of the subjects is relatively small, liver biopsy was not performed in most of the subjects and serum cytokine levels of the patients were not determined. However; the current study serves some clues suggesting that patients with chronic

HBV infection, even in the asymptomatic carrier status, may have an increased risk for alteration of bone metabolism. Therefore, we suppose that further studies are needed in this issue.

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