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• *BRIEF REPORTS* •

Reduced bone mineral density and altered bone turnover markers in patients with non-cirrhotic chronic hepatitis B or C infection

Ingolf Schiefke, Andreas Fach, Marcus Wiedmann, Andreas V. Aretin, Eva Schenker, Gudrun Borte, Manfred Wiese, Joachim Moessner

Ingolf Schiefke, Andreas Fach, Marcus Wiedmann, Andreas V. Aretin, Eva Schenker, Joachim Moessne, Department of Internal Medicine II
Gudrun Borte, Department of Radiology, University of Leipzig, Philipp-Rosenthal-Str. 27, 04103 Leipzig
Manfred Wiese, Department of Internal Medicine II, Municipal Hospital "St. Georg", Delitzscher Str. 141, 04129 Leipzig, Germany
Correspondence to: Ingolf Schiefke, M.D., Department of Medicine II, University of Leipzig, Philipp-Rosenthal Str. 27, 04103 Leipzig, Germany. schi@medizin.uni-leipzig.de
Telephone: +49-341-9712230 Fax: +49-341-9712239
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Abstract

AIM: Previous studies suggest that loss of bone mineral density (BMD) frequently occurs in patients with chronic viral liver disease, presenting with histologically proven liver cirrhosis. However, little is known about the occurrence of bone disease in non-cirrhotic patients with chronic hepatitis B or C. Therefore, it was the aim of this study to evaluate this particular population for BMD and bone turnover markers.

METHODS: Biochemical markers of bone turnover and BMD were measured in 43 consecutive patients with HCV (n = 30) or HBV (n = 13) infection without histological evidence for liver cirrhosis. Mean age was 49 years (range 26-77 years). BMD was measured by dual X-ray absorptiometry in the femoral neck (FN) and the lumbar spine (LS) region. In addition, bone metabolism markers were measured.

RESULTS: BMD was lowered in 25 (58%) of the patients with chronic hepatitis B or C (FN: 0.76 (0.53-0.99); LS: 0.96 (0.62-1.23) g/cm²). Eight (32%) osteopenic patients were diagnosed with osteoporosis. Bone-specific alkaline phosphatase (P = 0.005) and intact parathyroid hormone (iPTH) (P = 0.001) were significantly elevated in the more advanced stages of fibrosis. Mean T-score value was lower in patients with chronic hepatitis C as compared to patients suffering from chronic hepatitis B; however, the difference was not statistically significant (P = 0.09).

CONCLUSION: There was a significantly reduced BMD in non-cirrhotic patients with chronic hepatitis B or C infection. Alterations of bone metabolism already occurred in advanced liver fibrosis without cirrhosis. According to our results, these secondary effects of chronic viral hepatitis should be further investigated.

INTRODUCTION

Bone manifestations are well-known extrahepatic complications of chronic liver disease[1,2]. Reduced bone formation and low bone turnover are the likely factors contributing to the increased rate of bone loss observed in chronic liver disease[3-5]. The prevalence of osteoporosis among patients with chronic liver diseases ranges from 10% to 60%[3,4,6,7], the highest being observed in cholestatic liver disease and alcoholic liver disease. A recent study revealed that the prevalence of osteoporosis in patients with cirrhosis secondary to hepatitis B or C was nearly 50%[8]. A reduction in bone mineral density (BMD) leads to increased bone fragility, with osteopenia and osteoporosis leading to a two-fold and four- to five-fold increased risk for fracture, respectively[9,10].

Although, in general many risk factors can be identified for the pathogenesis of osteoporosis, other factors related to the specific gastrointestinal pathology in various liver disorders are still unknown. In particular, chronic cholestasis in patients with primary biliary cirrhosis[11,12], corticosteroid use[3,13], smoking[14], physical inactivity[15], alcohol abuse[16], malnutrition, and vitamin D deficiency[17,18] have been assumed as additional contributing factors for the deterioration of bone metabolism in chronic liver disease. However, the results of studies investigating the relative impact of these factors have not been consistent.

Only a few studies on bone formation have been performed in patients suffering from chronic hepatitis B (HBV), C (HCV), or D virus infection[8,19]. Some studies showed that cirrhotic patients with viral hepatitis display a prevalence of osteoporosis up to 53%[8]. In addition, combination therapy with interferon (IFN) and ribavirin for chronic hepatitis C[20] may affect bone turnover. Most studies of bone disease were performed in patients with cirrhosis. Nevertheless, little is known about the occurrence

of bone disease in non-cirrhotic patients with chronic hepatitis B or C.

Therefore, in the present study we evaluated the BMD and biochemical bone turnover markers in non-cirrhotic patients with chronic hepatitis B or C infection.

MATERIALS AND METHODS

Patients

Forty-three consecutive patients of our outpatient department with chronic HBV or HCV infection participated in this study. The group consisted of 12 men and 31 women with a median age of 49 years (range 26-77 years).

All patients had positive serological markers and a positive polymerase chain reaction (PCR) for HBV or HCV infection for more than 6 mo (13 infections were HBV and 30 HCV related).

The relevant demographic- and disease-related data at the time of enrollment are listed in Table 1. Cirrhotic patients were excluded, with cirrhosis detected by a liver biopsy within 6 mo prior to study entry, abdominal ultrasonography, and laboratory findings (Table 2). All liver biopsy specimens were analyzed according to the Knodell Histologic Activity Index (HAI) which classifies the inflammatory activity with a grading score from 0 to 18 and fibrosis progression with a staging score from 0 to 4[21].

Data about body weight and height, smoking habits, previous and present physical activity, menstrual status, number of pregnancies, and amount of coffee intake were obtained from a self-administered questionnaire and by extensive review of the patients' charts.

None of the patients had a family history of osteoporosis, nor did they receive calcium, vitamin D, corticosteroids, or any medication related to mineral metabolism. None of the 30 women had ever received hormone replacement therapy. All patients signed informed consent and the protocol was approved by the local human ethics committee.

Bone mineral density measurements

Bone mineral/mass density (BMD in g/cm^2) was assessed in all patients at the lumbar spine (LS; L1-L4) and femoral neck (FN) region using dual X-ray absorptiometry (DEXA) (Hologic QDR-1500, S/N 1535, Waltham, MA). The coefficient of variation for the BMD was 0.5%, both at the FN and LS region. Long-term stability of the instrument was assured by a daily scan of a spine phantom of known composition. All scans were carried out on the same machine by the same operator and were analyzed with the same software. The results of the patients' BMD measurements were compared with mean BMDs from age- and sex-matched controls from a large population database (Hologic) and expressed as standard deviation of the mean (*Z*-score) to avoid bias of age and sex. These reference data provided by the manufacturer have a good concordance with data derived from a previously described German control population[22]. Furthermore, osteopenia and osteoporosis were defined as BMD measurements between 1 and 2.5 ($-2.5 \leqslant T \leqslant 1$) and more than 2.5 ($T \leqslant -2.5$) standard deviations below the mean bone density for young adults, respectively, consistent with the World Health Organization criteria[23].

Care was taken not to measure collapsed lumbar vertebrae to avoid false BMD readings. Radiographs and bone scans were not routinely performed to look for asymptomatic bone fractures.

Biochemical measurements

Blood was drawn in the morning after overnight fasting. Serum was analyzed for calcium (Ca) (normal range 2.02-2.60 mmol/L s), ionized calcium (iCa) (normal range 1.12-1.23 mmol/L s), phosphate (normal range 0.89-1.45 mmol/L s), alkaline phosphatase (AlkPhos) (normal range 1.5-4.3 μmol/L s), bone-specific alkaline phosphatase (BALP) (normal range <2.5 μmol/L s), intact parathyroid hormone (iPTH) (normal range 1.06-6.89 pmol/L), 25-hydroxycholecalciferol [25 (OH)D_3] (normal range 10-68 pg/mL) and 1,25-dihydroxy-

Table 1 Baseline characteristics and biochemical markers in non-cirrhotic hepatitis B or C patients

Parameter	All patients (n = 43)	Hepatitis B (n = 13)	Hepatitis C (n = 30)
Age (yr)	49 (26–77)	49 (27–67)	49 (26–77)
BMI (kg/m^2)	23.32 (18.42–32.45)	23.33 (19.44–30.35)	23.32 (18.42–32.45)
Males, *n* (%)	12 (28)	6 (46)	6 (20)
Osteoporosis (%)	8 (19)	2 (15)	6 (20)
Osteopenia (%)	25 (58)	7 (54)	18 (60)
Smoking, *n* (%)	6 (14)	4 (31)	2 (7)
Diagnosis, *n* (%)		13 (30)	30 (70)
HBV-DNA (log copies/mL)		2.46 (1.00–7.70)	
HCV-RNA (log IU/mL)			5.46 (1.00–5.93)
Staging[1]			
Low grade 1–2	32 (82)	9 (82)	23 (82)
High grade 3–4	7 (18)	2 (18)	5 (18)
ALT (μmol/L s)	0.70 (0.14–1.58)	0.55 (0.44–1.39)	0.74 (0.14–1.58)
Albumin (g/L)	42.2 (38.7–46.8)	41.8 (39.8–46.8)	42.1 (38.7–45.5)

[1]Four patients had no biopsy in the last 2 yr.

Table 2 Characteristics of bone metabolism in patients with chronic viral liver disease

Parameter	All patients (n = 43)	Hepatitis B (n = 13)	Hepatitis C (n = 30)
AlkPhos (μmol/L s)	2.95 (1.32–10.87)	2.39 (1.96–6.67)	2.98 (1.32–10.87)
BALP (μmol/L s)	1.69 (0.76–3.89)	1.52 (0.76–2.94)	1.51 (0.76–3.89)
iPTH (pmol/L)	2.57 (1.35–5.96)	2.59 (1.35–5.96)	2.69 (1.45–5.38)
25 (OH)D_3 (pg/mL)	27.4 (11.9–65.7)	27.40 (15.40–60.20)	28.05 (11.90–65.70)
1,25 (OH)$_2D_3$ (pg/mL)	38.7 (10.0–86.8)	41.40 (21.50–60.7)	38.20 (10.00–86.80)
Phosphate (μmol/L)	1.08 (0.57–1.43)	0.95 (0.57–1.15)	1.09 (0.67–1.43)
Ca (mmol/L)	2.43 (2.17–2.58)	2.44 (2.22–2.58)	2.39 (2.17–2.57)
iCa (mmol/L)	1.23 (0.99–1.34)	1.22 (1.12–1.29)	1.25 (0.99–1.34)
PD/UCr (pmol/μmoL)	58.0 (30.0–235.0)	59.0 (30.0–102.0)	57.00 (34.00–235.00)
DPD/UCr (pmol/μmoL)	13.3 (6.3–50.5)	10.6 (6.3–26.7)	13.85 (6.4–50.5)

cholecalciferol [1,25$(OH_2)D_3$] level (normal range 20.2-46.2 pg/mL), alanine amino transferase (ALT) (normal range <0.67 μmol/L s), albumin (normal range 37-53 g/L), and prothrombin time activity (PT) (normal range >70%).

Creatinine (UCr), pyridinoline (PD) and desoxypyridinoline-crosslinks (DPD) were measured from the second morning urine or from 24-h collection specimens using standardized commercial assays. Urinary excretion was expressed as the ratio of PD and DPD to creatinine (normal range PD/UCr 40-100 pmol/μmoL; DPD/UCr 8-45 pmol/μmoL).

Hepatitis markers

Serum samples were stored at -30 ℃ until testing. Hepatitis B surface antigen (HBsAg), antibodies against hepatitis B surface antigen (anti-HBs) and hepatitis B core antigen (anti-HBc), and antibodies against hepatitis C virus (anti-HCV) were tested with commercial enzyme immunoassays (Abbott Diagnostics, Wiesbaden, Germany). HBV-DNA and HCV-RNA were measured by quantitative PCR (Abbott Diagnostics).

Statistical analysis

Data were summarized by median and range for continuous variables and frequency counts for categorical variables. Differences between groups were assessed using Fisher's exact test for binary variables, the Mann-Whitney *U* test and the Kruskal-Wallis test for continuous variables. A *P*-value of <0.05 was considered statistically significant.

RESULTS

Baseline data and BMD

The demographic- and disease-related data at the time of enrollment are listed in Tables 1 and 2. Median raw BMD values were 0.96 (0.62-1.23) for the LS and 0.76 (0.53-0.99) for the FN region, respectively. BMDs at all sites correlated with another (r = 0.78). The mean *T* scores were -1.04 (-4.13-1.26) at the LS and -1.52 (-3.65-0.32) at the FN region. At the time of enrollment 23 of 43 (51%) of the patients had evidence of osteopenia of the spine and femur. Osteoporosis of the spine was present in 3 of 43 (7%) of the patients, and 8 of 43 (19%) had osteoporosis of the FN.

When patients were classified according to the histological staging system, the BMD was lower in the more advanced stages of liver disease (P = 0.027) (Figure 1). In addition, we found a statistically significant difference between low (staging 0-2) and high (staging 3-4) stages of fibrosis (P = 0.025). Compared to an age-matched population (*Z*-score) we found a moderately decreased bone mass at the LS and FN region (pooled *Z*-score = -0.23), and median BMD was lower in patients with more advanced fibrosis (low stage -0.09 *vs* high stage -1.09) (Figure 2). Median values of BMD of FN and LS region did not differ between sexes (P = 0.57 and 0.78). None of the patients reported a past history or symptoms of bone fracture at the time of enrollment. BMD and *T*-scores were lower in patients with chronic hepatitis C than in patients with chronic hepatitis B, although this was not statistically significant (Figure 3).

Biochemical and hepatitis markers

As expected, the biochemical parameters of liver function did not differ significantly between chronic hepatitis B and C patients (Table 1) and were not related to sex or presence of osteoporosis. In all patients, phosphate, serum ionized calcium, iPTH, 1,25$(OH_2)D_3$, and 25$(OH)D_3$ were in the normal range (Tables 1 and 2).

However, BALP (P = 0.005) and iPTH (P = 0.001) were significantly elevated in the more advanced stages of fibrosis (Figures 4, 5). There was no significant difference between the different fibrosis stage groups in terms of serum ionized calcium, 1,25$(OH_2)D_3$, 25$(OH)D_3$, DP, or DPD excretion.

The correlation between the parameters of hepatic function and calcium-iPTH axis is shown in Tables 3 and 4. iPTH did not correlate with total or ionized calcium, creatinine, 1,25$(OH_2)D_3$ but with BMD (P = 0.02). We found no group difference in terms of serological hepatitis markers, viral load, or presence of osteoporosis.

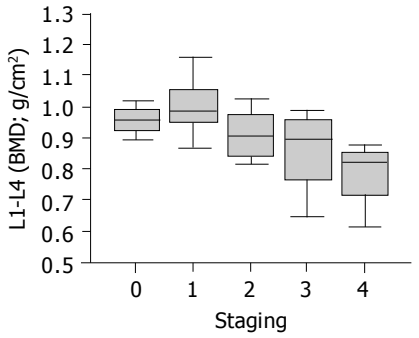

Figure 1 Diminished bone density in histologically proven advanced liver disease.

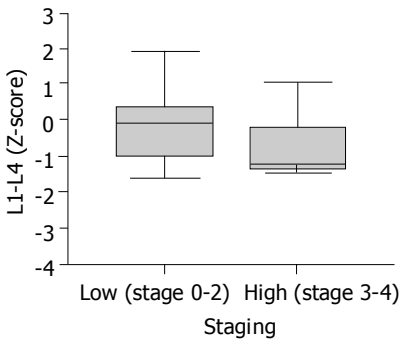

Figure 2 Reduced median *Z*-score in patients with higher staging in liver biopsy.

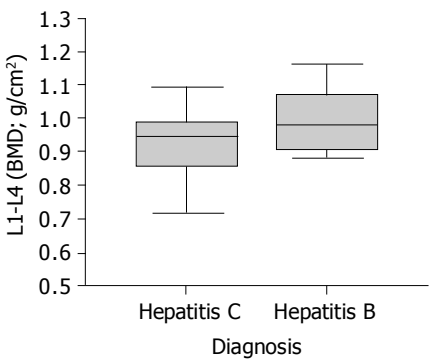

Figure 3 BMD in patients with chronic hepatitis B or C at LS.

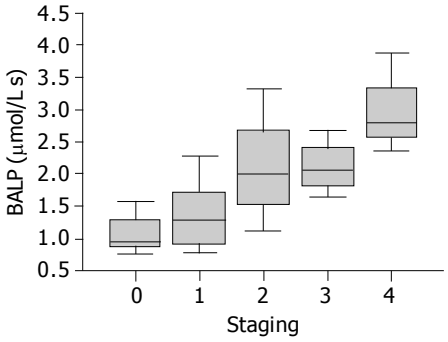

Figure 4 Increased BALP in advanced non-cirrhotic liver disease.

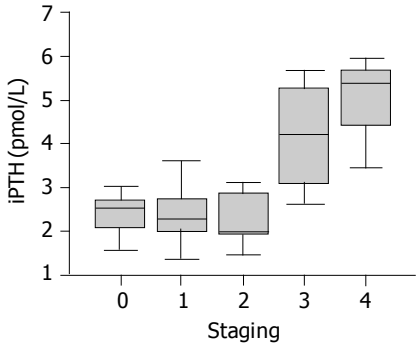

Figure 5 iPTH in patients with chronic viral hepatitis and different histological staging.

Table 3 Correlation of liver histology (staging) with bone markers in patients with chronic hepatitis B or C

Parameter	*r*	*P*
iPTH	0.523	0.001
25 $(OH)D_3$	–0.025	0.878
1,25 $(OH)_2D_3$	0.006	0.973
Phosphate	–0.033	0.841
iCa	–0.215	0.188
BALP	0.469	0.001

Table 4 Correlation of BMD femoral neck with bone markers and vitamin D in patients with chronic viral hepatitis

Parameter	*r*	*P*
iPTH	0.325	0.02
25 $(OH)D_3$	0.282	0.067
1,25 $(OH)_2D_3$	–0.071	0.649
Phosphate	–0.204	0.057
iCa	0.022	0.888
BALP	–0.082	0.599

DISCUSSION

Osteoporosis is a frequent complication of end-stage liver disease, irrespective of the etiology. The prevalence varies between 9% and 60%, the highest being observed in cholestatic disorders and alcoholic liver disease[4,6,7,16,24]. Cirrhotic patients show reduced osteoid thickness, osteoblast surface, and bone formation rate[25].

Our study of 43 patients reveals a high prevalence of osteopenia and osteoporosis in patients with non-cirrhotic viral hepatitis. In the present study, we provide data on changes in the calcium-BALP-iPTH axis and their clinical significance in a cohort of patients with non-cirrhotic chronic hepatitis B or C. Marked alteration of iPTH and BALP levels was detected in histologically proven more advanced disease, mainly reflecting the severity of fibrosis. Elevation of iPTH serum levels correlated with an increase of BALP. We show in our study that progressive increase of iPTH occurs in more advanced disease stages. Similar results were reported in previous studies of cirrhotic patients prior to orthotopic liver transplantation[13,26]. They found a high level of PTH and its degradation products, especially in PBC patients[13]. After liver transplantation, iPTH levels dropped significantly in the cholestatic liver disease group but not in the hepatitis group[13,27]. PTH is transmogrified by the liver into biologically active forms, and the detection of these active metabolites is crucial[28]. The present data suggest that elevated iPTH levels may be partially responsible for shifting the balance in bone metabolism towards bone resorption, even in younger patients with chronic hepatitis. As suggested by Duarte *et al*[18], the reduced clearance capacity for PTH metabolites in the liver may cause the elevation in advanced liver disease. We found a significant inverse correlation between iPTH levels and BMD at the FN region (Table 4). Clinically, the differentiation between spine and femur osteopenia or osteoporosis is not critical, as the presence of either is treated similarly. However, it should be noted that non-liver-specific factors are probably more relevant in bone turnover.

Besides markers of bone formation (i.e., BALP), objective resorption markers (i.e., urine pyridinoline) were also measured in this study of non-cirrhotic viral hepatitis patients. Fasting urinary/creatine ratio of the collagen-crosslinks (particularly desoxypyridinoline) has been shown to be the most specific currently available biochemical marker of bone resorption[29,30]. However, neither PD/UCr nor DPD/UCr ratio was significantly elevated in our patients, which might suggest that these patients do not exhibit increase of bone resorption. But the excretion of PD/UCr and DPD/UCr tended to be at the upper limit of normal, and the observed lack of a significant difference may reflect the relatively small number of patients studied. There was no inverse correlation between these variables and bone density. In patients with viral chronic hepatitis and osteodystrophy, increased levels of urinary PD and DPD were found[31], which inversely correlated with BMD, suggesting increased osteolysis. In addition, patients with viral cirrhosis showed elevated DPD values[8]. Similarly, urinary hydroxyproline excretion is also often used as an indirect measure of bone matrix resorption[30,32]. In patients with alcohol-related liver disease mean urinary excretion of hydroxyproline was abnormally high. The increase in urinary hydroxyproline was probably caused by active hepatic fibrogenesis and collagen remodeling in the sick liver, which could explain the observed changes in our patients.

In conclusion, this study describes for the first time an association between non-cirrhotic viral hepatitis and osteopenia/osteoporosis. Non-cirrhotic chronic hepatitis B or C patients showed alterations in calciotropic hormones and bone metabolism leading to a decreased BMD. Therefore, further studies with the aim to identify biochemical markers to characterize particular metabolic changes are warranted and required to confirm this association. Our data suggest that an iPTH increase results in a BALP increase without further evidence of a high-turnover bone loss. Larger studies with frequently repeated clinical measurements or more sensitive biochemical or laboratory measures of disease activity, performed over a course of many months or years, may be necessary to determine the precise contribution of disease stage on BMD. Patients should be considered for routine bone densitometric assessment and, if necessary, anti-osteoporotic therapy.

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