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• *BRIEF REPORTS* •

Is osteoporosis a peculiar association with primary biliary cirrhosis?

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Abstract

AIM: (1) To compare the prevalence of osteoporosis (*t*-score ⩽-2.5 SD) between stage IV PBC patients, and two groups of age- and sex-matched controls: one with hepatitis C virus (HCV)-related cirrhosis, and the other one consisting of a group of healthy subjects from the general population; (2) to identify the main risk factors for the development of bone loss.

METHODS: Thirty-five stage IV PBC patients (mean age 52.5±10 years), 49 females with HCV-related cirrhosis (mean age 52.9±5.8 years) and 33 healthy females (mean age 51.8±2.22 years) were enrolled in the study. Bone metabolism was evaluated by measuring serum calcium corrected for serum albumin (Ca corr.), 25-hydroxy vitamin D (25-OH vit D), parathyroid hormone, osteocalcin. Bone mineral density (BMD) was assessed at the lumbar spine by dual-photon X-ray absorptiometry.

RESULTS: Osteoporosis was present in 5/35 PBC patients (14.2%) and in 7/49 HCV-related cirrhotic patients (14.3%), without any statistical difference between the two groups. Among healthy control subjects, none had osteoporosis. No difference was found between the three groups in serum parameters of bone metabolism. Univariate analysis showed that menopausal state and low BMI were significantly correlated with osteoporosis. Multivariate regression analysis showed that menopausal status, BMI <23, and old age were independent variables significantly correlated with osteoporosis.

CONCLUSION: PBC in itself has no negative influence on BMD. End-stage liver disease patients carry a disease-specific risk for osteoporosis, but have an effective risk of bone loss in relation to individual potential risk for each patient. A practical message should be taken into account, that is, every effort should be made to prevent osteoporosis when a patient has simple osteopenia, or if it is a woman in or near menopausal age.

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Key words: Osteoporosis; Primary biliary cirrhosis; HCV infection

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INTRODUCTION

Osteopenia is a major complication of end-stage liver disease and a real risk in liver transplant patients[1]. It is also a common clinical problem, however, in association with chronic cholestatic liver disease, particularly PBC, due to predominant female sex and older age[2]. Osteoporosis was recently found in 20% of 176 patients with PBC, occurring 32 times more frequently than expected[3]. Despite the wisdom that osteoporosis is common in PBC than in other types of liver disease, new data are emerging in the literature concerning this field.

In a previous study, including cirrhotic patients evaluated for liver transplantation, we failed to find any significant difference in bone mineral density (BMD) between patients with chronic cholestatic liver disease and cirrhotics of various etiology[4]. Newton *et al*., retrospectively reviewed a large group of PBC patients in Newcastle (UK) and found osteoporosis in 31.2% of cases; none of the patients went on to develop *de novo* osteoporosis during a mean follow-up of 10.1 years[5]. The authors concluded that osteoporosis is not a specific complication of PBC. They had not considered age- and sex-matched controls from the general population or control groups with different types of liver disease. It should also be stressed that PBC is a heterogeneous disease, and usually any PBC study population includes patients with different severity of liver damage ranging from early disease to cirrhotic stage.

The aims of the present study were therefore: (1) to compare the prevalence of osteoporosis between stage IV PBC patients and two groups of age- and sex-matched controls: one with hepatitis C virus (HCV)-related cirrhosis, and the other one consisting of healthy subjects from general population; (2) to identify the main risk factors for osteoporosis.

MATERIALS AND METHODS

Patients

Thirty-five consecutive female stage IV PBC patients (mean

age 52.5±10 years), 49 females with HCV-related cirrhosis (mean age 52.9±5.8 years) and 33 female healthy subjects (mean age 51.8±2.22 years) were enrolled in the study. The clinical characteristics of the three groups are summarized in Table 1. Body mass index was significantly lower in both PBC and HCV-related cirrhosis patients compared to healthy controls ($P<0.05$). All subjects were asymptomatic for bone loss, and none were taking calcium and/or vitamin D supplementation. None of the patients were treated with steroids. Patients with HCV-related cirrhosis have not been previously treated with interferon/ribavirin therapy. The patients were not given hormone replacement. PBC patients were treated with ursodeoxycholic acid [12-15 mg/(kg·d)] twice a day after meals.

The diagnosis of PBC was obtained according to clinical, immunological, and histological grounds. All the patients with PBC has histological stage IV, according to Scheuer's classification[6]. The diagnosis of HCV-related cirrhosis was obtained by clinical, histological, and virological methods, including serum positivity for HCV-RNA by means of PCR assay.

None of the patients were ingesting alcohol. Ethical approval was obtained from the hospital's ethics committee and informed consent was obtained from each patient, prior to their inclusion in the study.

Table 1 Clinical details of the patients

	PBC	HCV+	Control group
Number of patients	35	49	33
Age (mean±SD), yr	52.5±10	52.9±5.8	51.8±2.22
Age at menopause (mean±SD), yr	48.3±4.5	48.5±4.9	47±7.46
BMI (mean±SD)	22.8±3.2[a]	23.4±4.2[a]	25.9±4.29

[a]$P<0.05$ *vs* control group.

Bone density measurement

BMD was assessed by DEXA (Hologic QDR 4500 C, Walthom, MA, USA) at lumbar spine (L2-L4). The manufacturer's reference database was obtained by measuring more than 500 healthy male volunteers at the University of California (San Diego, CA) and at other QDR-1000 installations across the USA. Reference data collected on Hologic systems in Europe, including Italy, have demonstrated a close correlation with US values. In our hands, the long-term (1 year) coefficient of variation *in vitro* with a Hologic phantom is 0.44%; the short-term (1 wk) coefficient of variation *in vivo*, with five consecutive measurements performed in 10 healthy volunteers, is 1.2%. Osteoporosis was defined according to WHO in terms of *t*-score lower than -2.5 SD. The *t*-score was defined as the number of SD above or below the mean BMD for young adult mean value. The *z*-score for BMD was defined as the number of SD above or below the mean BMD for age- and sex-matched normal population.

Biochemical parameters

The following parameters were assessed in all subjects: serum calcium, parathyroid hormone (PTH), osteocalcin (BGP), 25-hydroxy vitamin D (25-OHD). Serum Ca was measured by common automated laboratory methods. Serum calcium corrected for albumin concentration was calculated using the following formula:

Ca corr (mg/dL) = Ca tot (mg/dL)+4-albumin (g/L)

The result was divided by 4 and expressed in mmol/L.

PTH was tested by IRMA (Allegro TM Intact, Nichols Institute, S. Juan Capistrano, CA, USA). This method detects the biologically intact 1-84 amino acid chain of PTH. The normal range is 10-55 ng/L. The coefficient of variation was 3.6% intra-assay and 5.8% inter-assay. BGP was assayed by IRMA (Cis Bio International, Italy). The normal range is 4-26 mg/L. The coefficient of variation was 3.1% intra-assay, and 5.7% inter-assay. 25-OHD was determined by RIA (Incstar Corp, Stillwater, MN, USA). The normal range is 25-150 nmol/L. The coefficient of variation was 5.1% intra-assay and 9.7% inter-assay.

Statistical analysis

Results were expressed as the mean±SD. Student's *t*-test for unpaired data and the linear regression test were used, as appropriate. Logistic regression analysis was used to establish in which characteristics were independently associated with osteoporosis (age, menopausal status, BMI, 25-OHD, PTH, BGP). A P value $\leqslant 0.05$ was considered significant. Analyses were performed with the EPI-Info 6.04 and the Statistical Package for the Social Sciences (SPSS 10.0).

RESULTS

Osteoporosis was present in 5/35 PBC patients (14.3%) and in 7/49 HCV-related cirrhotic patients (14.3%), without any statistical difference between the two groups. No subjects with osteoporosis were identified in healthy female controls. The mean age of patients with osteoporosis was 54.2±55 years, and the mean age of those without osteoporosis was 52.5±8.4 years (P = NS). No difference was found between the three groups in s-Ca. The mean BMD at lumbar spine was lower in both PBC and HCV-related cirrhosis groups compared to healthy controls, without any statistical significance (Table 2). However, *t*-score was significantly lower in PBC group compared to either HCV-related cirrhosis group or control group (-1.55±0.99 in PBC *vs* -0.39±2.65 in HCV+ *vs* -0.29±0.98 in healthy controls, $P<0.05$).

Table 2 Bone metabolism in PBC, in HCV+ patients and in healthy controls

	PBC	HCV+	Controls
BMD (lumbar spine)	0.810±0.130	0.860±0.140	0.972±0.128
t-score ⩽–2.5 SD	–1.55±0.99[a]	–0.39±2.65	–0.29±0.98
z-score	–0.94±1.10[c]	–0.52±1.23	–0.82±1.02
25-OHD (nmol/L)	103.48±58.85[e]	98.38±44.65[g]	49.66±19.72
BGP (mg/L)	8.80±8.7	6.43±11.14	9.56±2.92
Intact (1–84) PTH (ng/L)	39.8±26.3	35±29.78	33.60±11.77
Ca corr albumin (mg/dL)	2.27±0.26	2.32±0.13	2.31±0.14

[a]$P<0.05$ *vs* controls. [c]$P<0.05$ *vs* HCV+. [e]$P<0.05$ *vs* controls. [g]$P<0.05$ *vs* controls.

Serum 25-OH vit D was significantly higher in both PBC group and HCV+ group *vs* healthy controls (103.48±58.85 nmol/L in PBC *vs* 98.38±44.65 in HCV+ *vs* 49.66±19.72 in healthy controls, $P<0.005$). No significant differences in serum BGP and PTH were found between the three groups. Univariate analysis showed that menopausal state and low BMI were significantly correlated with osteoporosis (Table 3). Multivariate regression analysis showed that menopausal status, BMI <23, and advanced age were independent variables significantly correlated with osteoporosis (Table 4).

Table 3 Univariate analysis

Variable	*n*	Osteoporosis		*P*
		n	%	
Menopausal status				
Yes	46	10	(21.7)	
No	38	2	(5.2)	0.032
Disease				
HCV	49	7	(14.3)	
PBC	35	5	(14.3)	1.000
Normal 25-OH vit D				
Yes	67	10	(14.9)	
No	17	2	(11.8)	0.739
Normal BGP				
Yes	18	4	(22.2)	
No	66	8	(12.1)	0.278
Serum PTH				
Normal	48	5	(10.4)	
High	36	7	(19.4)	0.242
BMI >23				
Yes	56	4	(7.1)	
No	28	8	(25.8)	0.008

Table 4 Multivariate analysis

Variable	Adjusted OR	95.0%CI		*P*
		Low	Upper	
Disease (HCV *vs* PBC)	0.429	0.086	2.149	0.304
BGP	1.785	0.323	9.866	0.507
25-OH vit D	0.674	0.098	4.609	0.687
PTH	2.050	0.494	8.501	0.323
Menopausal status	23.102	1.524	350.186	0.024
BMI <23	5.703	1.228	26.488	0.026
Age (yr)	1.151	1.004	1.320	0.044

DISCUSSION

Our results indicate that female patients with end-stage PBC have a similar percentage of osteoporosis than sex- and age-matched controls with HCV-related cirrhosis. However, PBC patients had significantly lower *t*-score compared to age- and sex-matched controls from general population. All types of liver disease are acknowledged and some osteopenia risk factors are common, e.g. cirrhosis, female sex, old age, alcohol consumption, hypogonadism, steroid therapy, impaired conversion to 25-OH vit D, low BMI, vitamin D receptor (VDR) polymorphism, reduced BGP activity[7-14], but certain specific risk factors for osteoporosis exist in chronic cholestatic liver disease, i.e. calcium and vitamin D malabsorption, hyperbilirubinemia, and cholestyramine therapy[15,16]. In our patients, however, vit D status, evaluated by 25-OH vit D serum levels, was similar in the three groups of subjects, as well as serum Ca, and serum BGP. Furthermore, no secondary hyperparathyroidism was found in our cases, suggesting a normal intestinal calcium absorption.

In order to avoid confounding factors such as corticosteroids and alcohol consumption, we have chosen three groups of subjects with the same demographic characteristics (same geographical area, same prevalence of menopausal status, same age) without evidence of bone disease, and without calcium and/or vitamin D supplementation. Multivariate analysis showed that menopausal status, BMI <23, and old age were independent variables significantly correlated with osteoporosis. This finding apparently confirms previous results[4,11,17] in which chronic liver disease *per se*, and not a specific etiology, should be responsible for hepatic osteodystrophy. Osteoporosis has been shown to be significantly associated with postmenopausal status in several studies[18,19]; however, all PBC series include patients with different histological stages. In our study, we included only patients with advanced disease, namely cirrhotic patients.

As far as our results are concerned, the univariate analysis (which included the type of liver disease, menopausal status, 25-OHD, BGP, PTH, BMI >23) showed that both menopause and old age were associated to bone loss in both groups. We did not assay VDR gene polymorphism in these study groups, but there is evidence that VDR genotypes predict lower BMD in general population in different countries[20,21]. As far as VDR gene polymorphism in liver disease is concerned, there is evidence that similar results may be extended also in PBC[9,22]; however, in our knowledge, no other groups of liver disease patients have been tested for VDR gene polymorphism.

Nevertheless, the mean values of serum parameters of bone metabolism (25-OH vit D, BGP, PTH, and Ca corr) in both PBC and HCV-related cirrhosis patients did not show any difference compared to controls. These data suggest that osteodystrophy in end-stage liver disease patients is characterized by a low turnover osteoporosis.

BGP is a bone matrix protein synthesized by osteoblasts is vitamin K-dependent, but we failed to find any correlation between BGP and vitamin K activity measured by prothrombin activity (data not shown). Moreover, no difference was found in prothrombin activity between the two groups of cirrhotic patients.

PTH was assayed by RIA, using an antiserum directed against the mid-region of the hormone. When intact 1-84 PTH was assayed in cirrhotic patients, normal values of PTH levels were found.

In conclusion, PBC in itself has no negative influence on BMD. End-stage liver disease patients carry a disease-specific risk for osteoporosis, but have an effective risk of bone loss in relation to individual potential risk for each patient. A practical message should be taken into account, that is, every effort should be made to prevent osteoporosis when a patient has simple osteopenia, or if it is a woman in or near menopausal age.

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