Risk factors for osteoporosis in patients with end-stage liver disease

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Abstract. Patients with end-stage liver disease (ESLD) were evaluated and their clinical features were compared with the aim of identifying risk factors for osteoporosis. Seventy-nine patients with ESLD were enrolled in the current study. Bone mineral density (BMD) was assessed by dual-energy X-ray absorptiometry and compared with clinical features in patients with ESLD. BMD was identified to be significantly correlated with body mass index (r=0.430; P=0.001) and inversely correlated with total bile acid (r=-0.228; P=0.049) and urine N-telopeptide type I collagen/creatinine ratio (r=-0.280; P=0.024). Patients with osteoporosis were significantly older (osteoporosis vs. no osteoporosis, 63.0 vs. 56.0 years; P<0.05) and had higher values for total bile acid (osteoporosis vs. no osteoporosis, 306.0 vs. 129.1 μmol/l; P<0.05) and corrected calcium [osteoporosis vs. no osteoporosis, 9.85 (8.7-10.7) vs. 9.70 (8.8-10.6) mg/dl; P<0.05]. In multivariate analysis, age (β=-0.015±0.06; P=0.009) and total bile acid (β=-0.001±0.0001; P=0.041) were identified as independent factors for osteoporosis. Finally, the risk score for osteoporosis was defined as follows: Risk score=1.78-0.001 x total bile acid-(0.16 x age). The area under the receiver operating characteristic (ROC) curve risk score for osteoporosis is 0.778. Thus, the risk scores calculated in the present study may be used to predict osteoporosis in patients with ESLD.

Introduction

Osteoporosis is a generalized skeletal disease characterized by increased bone resorption and decreased bone formation, and is associated with an increased risk of bone fractures. The

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prevalence of osteoporosis has been increasing in Japan (1,2). Osteoporosis is classified as primary, secondary and idiopathic, with the term hepatic osteodystrophy first being defined in 1960 and including secondary osteoporosis (3). The prevalence of bone disease in patients with liver disease is higher than in the general population (4,5), as the majority of patients with chronic liver disease exhibit multiple risk factors for osteodystrophy, such as protein-calorie malnutrition and vitamin D deficiency. Osteoporosis is common in patients with cirrhosis, biliary disease (6,7) and alcoholism (8). However, hepatic osteodystrophy has gained little attention, as, compared with hepatocellular carcinoma and esophageal varices, it does not significantly affect life expectancy.

Advances in medical technology have prolonged life expectancy in patients with liver diseases. Osteoporosis decreases the quality of life, as it causes an increased risk of bone fractures. Therefore, hepatic osteodystrophy is considered to be a subject of interest.

In Japan, there have been few studies on osteoporosis in patients with end-stage liver disease (ESLD). Therefore, the clinical features of osteoporosis were examined in patients with ESLD and the associated risk factors were identified.

Materials and methods

Patient characteristics. The present study included 79 patients with ESLD admitted to Nagasaki University Hospital (Nagasaki, Japan) between June 2008 and October 2012. ESLD was defined as a patient who registered as a recipient of liver transplantation at Nagasaki University. Furthermore, patients were eligible to take part if they were aged ≥18 years and had ESLD. Patients who were administered medication for osteoporosis were excluded. The current study included 2 patients with Child-Pugh class A; however, these patients exhibited portal hypertension and experienced difficulties during therapy for bleeding varices. The etiology of chronic liver disease was based on clinical, analytical and radiological criteria. The current cohort included 40 males (50.6%) with a mean age of 57.0 years (range 24-75 years). The median Child-Pugh score was 10.0 and the model for ESLD (MELD) score was 14.00. All patients provided informed consent, and the study protocol conformed to the guidelines of the

Table I. Baseline characteristics of the patients (n=79).

Variables	Value
Age, years	57.0 (24-75)
Gender	
Male n (%)	40 (50.6)
Female n(%)	39 (49.4)
Etiology, n (%)	
Viral	51 (64)
HCV	38 (47)
HBV	13 (17)
Alcohol	10 (13)
PBC	7 (9)
AIH	4 (5)
PSC	3 (3)
NASH	2 (3)
Other	2 (3)
Body mass index	24.7
MELD score	14
Child-Pugh score	10
Diabetes, n (%)	33 (41)
HCC, n (%)	35 (44)
Compression fracture, n (%)	17 (21.5)
Osteoporosis, n (%)	23/79 (29.1)
Male, n (%)	7/40 (17.5)
Female, n (%)	16/39 (41.0)
AST (IU/l)	37.0 (12-154)
ALT (IU/l)	41.0 (19-244)
ALP (IU/l)	415.0 (171-1180)
BTR (μmol/l)	3.00 (1.58-7.24)
ChE (IU/l)	102.7 (36-279)
Total bile acid (μmol/l)	145.1 (7.4-639.5)
Albumin (g/dl)	2.80 (1.9-4.2)
T-Cho (mg/dl)	123.0 (50-331)
Bone-ALP (U/l)	24.9 (7.9-44.2)
Osteocalcin (ng/ml)	5.60 (1-32)
Urine-NTx/CR (nmol BCE/mmol CR)	37.3 (11.3-142.4)
Hemoglobin (g/dl)	10.6 (6.2-17.3)
Platelet ($10^4/\mu$l)	6.20 (2.2-27.3)
BMD (T-score) spine	-1.90 (-5.5-4.3)

HCV, hepatitis C virus; HBV, hepatitis B virus; PBC, primary biliary cirrhosis; AIH, auto-immune hepatitis; PSC, primary sclerosing cholangitis; NASH, non-alcoholic steatohepatitis; MELD score, model for end-stage liver disease; HCC, hepatocellular carcinoma; AST, aspartate transaminase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BTR, branched chain amino acid to tyrosine ratio; ChE, cholinesterase; T-Cho, total cholesterol; BMD, bone mineral density; NTx, N-telopeptide type I collagen; BCE, bone collagen equivalent; CR, creatinine.

Declaration of Helsinki and was approved by the Nagasaki University Ethics Committee (approval no. 16042537). Patient characteristics are summarized in Table I.

Table II. Correlation between clinical data and bone mineral density.

Variables	Correlation coefficient	P-value
Age	0.164	0.149
Body mass index	0.430	0.001
MELD score	-0.019	0.880
Child-Pugh score	-0.153	0.205
AST	0.051	0.656
ALT	-0.007	0.954
ALP	-0.082	0.474
BTR	-0.117	0.327
ChE	0.160	0.159
Total bile acid	-0.228	0.049
Albumin	0.128	0.263
T-Cho	0.048	0.677
Hemoglobin	0.109	0.337
Platelet	-0.076	0.503
Prothrombin time INR	-0.016	0.893

MELD score, model for end-stage liver disease; AST, aspartate transaminase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BTR, branched chain amino acid to tyrosine ratio; ChE, cholinesterase; T-Cho, total cholesterol.

Table III. Correlation between biochemical bone metabolism data and bone mineral density.

Variables	Correlation coefficient	P-value
Urine NTx/CR	-0.280	0.024
BAP	0.024	0.841
Osteocalcin	0.052	0.673
Corrected calcium	-0.109	0.340
Intact PTH	-0.127	0.289
1,25(OH)2 vitamin D	0.155	0.201

NTx, N-telopeptide type I collagen; CR, creatinine; BAP, bone specific alkaline phosphatase; PTH, parathyroid hormone.

The following clinical items were collected at enrolment for each patient: Age, gender, body mass index (BMI), Child-Pugh score, MELD score, and treatments that could affect bone mineral density (BMD; corticosteroids, hormone replacement therapy, calcium and vitamin supplementation, and antiosteoporotic agents, taken either currently or in the last 24 months).

In addition, the results of recent blood tests (within 1 month) were collected: Serum bilirubin, alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase, γ-glutamyl transferase, platelet count, prothrombin time, albumin, total cholesterol and total bile acid.

Assessment of bone mineral density. BMD was assessed by dual-energy X-ray absorptiometry (DEXA) using a Norland

Table IV. Clinical, biological and biochemical data in patients with and without osteoporosis.

Variables	Osteoporosis (23/79)	No osteoporosis (56/79)	P-value
Age (years)	63.0 (24-75)	56.0 (34-70)	<0.05
Gender (male/female)	7/16	33/23	<0.05
BMI (kg/m^2)	23.8 (18.4-30.2)	25.3 (18.6-37.3)	N.S
MELD score	14.5 (9-29)	14.0 (6-34)	N.S
Child-Pugh score	10.0 (6-13)	9.0 (6-15)	N.S
Diabetes (%)[a]	34.8	30.4	N.S
HCC (%)[a]	47.8	41.1	N.S
AST (IU/l)	38.0 (14-128)	34.0 (12-154)	N.S
ALT (IU/l)	35.0 (1-56)	44.0 (9-244)	N.S
ALP (IU/l)	406.0 (178-46)	415.0 (171-1185)	N.S
BTR (μmol/l)	2.81 (1.54-8.17)	3.07 (0.76-36.90)	N.S
ChE (IU/l)	94.0 (36-179)	91.5 (33-279)	N.S
Total bile acid (μmol/l)	306.0 (28.4-639.5)	129.1 (7.2-631)	<0.05
Albumin (g/dl)	2.7 (2.0-3.7)	2.9 (1.9-4.2)	N.S
T-Cho (mg/dl)	105.0 (50-171)	123.0 (51-331)	N.S
Hemoglobin (g/dl)	10.6 (7.9-14.1)	10.7 (6.2-17.3)	N.S
Platelet (10^4/μl)	6.4 (2.1-26.3)	6.0 (0.2-27.3)	N.S
Prothrombin time INR	1.41 (1.24-1.81)	1.43 (0.99-3.28)	N.S
Compression fracture (%)	60.9	5.4	<0.05

[a]Data analyzed by χ^2 test. Remaining analysis was conducted using the Mann-Whitney U test. BMI, body mass index; N.S, not significant; MELD, model for end-stage liver disease; HCC, hepatocellular carcinoma; AST, aspartate transaminase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BTR, branched chain amino acid to tyrosine ratio; ChE, cholinesterase; T-Cho, total cholesterol; BMD, bone mineral density; NTx, N-telopeptide type I collagen; CR, creatinine.

XR-46 DEXA (Swissray Global Healthcare Holding Ltd., Taipei, Taiwan) scan of the lumbar spine. Norland XR-46™ software was used to compute the BMD in mg/cm^2 (of calcium hydroxyapatite equivalent), aggregate the results, and compute the mean BMD and the T score. The T score was defined as the number of standard deviations from the mean BMD values of healthy young adults. Hepatic osteodystrophy was defined as the presence of osteoporosis or osteopenia; osteopenia was defined as a T score between -1 and -2.5, and osteoporosis was defined as a T score <-2.5 below the reference value, or the existence of a compression fracture with osteopenia (T score: World Health Organization criteria) (9).

Statistical analysis. All statistical analysis was performed with Stat-flex version 6 (Artech Co., Ltd., Osaka, Japan). The Mann-Whitney U test was used for comparisons between groups. The presence of osteoporosis in ESLD was analyzed with a χ^2-test. Receiver operating characteristic (ROC) curves were used to determine an appropriate cut-off for the continuous variable and P<0.05 was considered to indicate a statistically significant difference.

Results

Correlation between clinical features and bone mineral density. The T-score was significantly correlated with BMI (r=0.430; P=0.001) and inversely correlated with total bile acid (r=-0.228; P=0.049; Table II). A significant correlation between the T-score and biochemical bone metabolism factors was only identified for the urine N-telopeptide type I collagen (NTx)/creatinine (CR) ratio (r=-0.280; P=0.024; Table III).

Clinical features in patients with and without osteoporosis. Osteoporosis was observed in 23 of 79 (29%) patients. Patients with osteoporosis were significantly older (osteoporosis vs. no osteoporosis: 63.0 (24-75) vs. 56.0 (34-70) years; P<0.05), had higher total bile acid (osteoporosis vs. no osteoporosis: 306.0 (28.4-639.5) vs. 129.1 (7.2-631) μmol/l; P<0.05) (Table IV) and higher corrected calcium [osteoporosis vs. no osteoporosis: 9.85 (8.7-10.7) vs. 9.70 (8.8-10.6) mg/dl ; P<0.05] (Table V).

An increased number of females had osteoporosis (osteoporosis vs. no osteoporosis, 69 vs. 41%; P<0.05) and the prevalence of bone fractures was significantly higher in patients with osteoporosis. Prevalence of osteoporosis tended to be higher in cholestatic disease than in non-cholestatic disease, although the difference was not significant [4/10 (40%) vs. 19/69 (27.5%); P=0.07]. Among the biochemical bone metabolism markers, only corrected calcium was significantly higher in patients with osteoporosis. In multivariate analysis, age (β=-0.015±0.06; P=0.009) and total bile acid (β=-0.001±0.0001; P=0.041) were independent risk factors for osteoporosis (Table VI).

Among patients with hepatitis C virus, those with osteoporosis were also significantly older [osteoporosis vs. no osteoporosis: 60.7 (52-72) vs. 55.5 years (43-70); P<0.05] (Table VII). They were more likely to be female (male

Table V. Biochemical bone metabolism data in patients with and without osteoporosis.

Variables	Osteoporosis (23/79)	No osteoporosis (56/79)	P-value
Urine NTx CR (nmol BCE/mmol CR)	47.2 (15.1-152.4)	35.6 (11.3-98.3)	N.S
BAP (μg/l)	25.3 (7.9-42.7)	24.5 (11.8-91.0)	N.S
Osteocalcin (ng/ml)	5.6 (1-14)	5.6 (1-32)	N.S
Corrected calcium (mg/dl)	9.85 (8.7-10.7)	9.70 (8.8-10.6)	<0.05
Intact PTH (pg/dl)	29.0 (14.0-93.0)	26.0 (7.0-90.0)	N.S
1,25(OH)2 vitamin D (pg/ml)	37.4 (16.0-67.0)	36.9 (10.4-89.5)	N.S

NTx, N-telopeptide type I collagen; BCE, bone collagen equivalent; CR, creatinine; N.S, not significant; BAP, bone specific alkaline phosphatase; PTH, parathyroid hormone.

Table VI. Independent risk factors for osteoporosis in patients with end stage liver disease.

Variables	P-value	β
Gender	0.362	0.096±0.105
Age	0.009	-0.015±0.006
Total bile acid	0.041	-0.001±0.0001
Corrected calcium	0.116	-0.199±0.125

Table VII. Clinical data in HCV patients with and without osteoporosis (n=38).

Variables	Osteoporosis (n=13)	No osteoporosis (n=25)	P-value
Age	60.7 (52-72)	55.5 (43-70)	<0.05
Gender			
M/f[a]	4/9	17/8	<0.05
Total bile acid	321.1 (41.2-639.5)	200.8 (17.1-631)	<0.05

[a]Data analyzed by χ^2 test. Remaining analysis was conducted using the Mann-Whitney U test. M, male; f, female.

Table VIII. Clinical data in cholestatic disease patients with and without osteoporosis (n=10).

Variables	Osteoporosis (n=4)	No osteoporosis (n=6)	P-value
Age	52.5 (24-65)	57.6 (43-70)	N.S
Gender			
M/f[a]	0/4	2/4	N.S
Total bile acid	305.7 (147.5-387.1)	123.2 (67.3-212.2)	<0.05

[a]Data analyzed by χ^2 test. Remaining analysis was conducted using the Mann-Whitney U test. M, male; f, female.

vs. female: 69 vs. 32%; P<0.05) and had higher total bile acid levels [osteoporosis vs. no osteoporosis: 306.0 (28.4-639.5) vs. 129.1 (7.2-631) μmol/l; P<0.05]. Among patients with cholestatic disease, those with osteoporosis had higher total bile acid levels [osteoporosis vs. no osteoporosis: 305.7 (147.5-387.1) vs. 123.2 (67.3-212.2) μmol/l; P<0.05] (Table VIII).

Risk score for osteoporosis. The risk score for osteoporosis was defined based on multivariate analysis as follows: Risk score=1.78 - 0.001 x total bile acid - (0.16 x age). The area under the ROC curve was 0.778.

Discussion

The present study revealed that BMD was significantly correlated with BMI and total bile acid. Previous studies reported that a low BMI and malnutrition were risk factors for osteoporosis and bone fractures (10,11). Low BMI was associated with malnutrition; therefore, in patients with ESLD, malnutrition was considered to be a factor as important as osteodystrophy.

The elevation of total bile acid is associated with the impairment of enterohepatic circulation (12). In the current study, total bile acid showed an inverse correlation with BMD in patients with ESLD. A significant difference between patients with and without osteoporosis was also found for total bile acid. In addition, significant differences were identified in total bile acid levels in patients with chronic hepatitis C and cholestatic disease. The mechanism is as follows: The impairment of bile acid secretion in the intestine decreases the formation of lipid soluble vitamins, including vitamin D (13,14). The activated vitamin D regulates calcium absorption and BMD. Furthermore, previous reports have shown that bile duct ligated rats had severe cholestasis, but developed low turnover osteoporosis (15).

Among biochemical bone metabolism markers, only urine NTx/CR was significantly negatively correlated with BMD. Among bone resorption markers, urine NTx/CR is the most frequently used in clinical practice (16). This biochemical bone metabolism marker changes prior to alterations in BMD. Therefore, urine NTx/CR may be an effective marker of BMD in patients with ESLD. However, osteogenic markers, such as osteocalcin and bone specific alkaline phosphatase were not correlated with BMD. This indicated that bone resorption was greater in patients with ESLD.

In the present study, the prevalence of osteoporosis was 29% (17.5% in males and 41.0% in females). According to the Japanese Society for Bone and Mineral Research, the prevalence of osteoporosis in the Japanese general population is 4% in males and 24% in females (17). Therefore, ESLD is a major risk factor for osteoporosis. The observed prevalence in patients with ESLD was consistent with previous reports (18-20).

Previous studies have reported that cholestatic liver disease, including primary biliary cirrhosis and primary sclerosing cholangitis are risk factors for osteoporosis (21-23). However, in the current study, the prevalence of osteoporosis tended to be higher in cholestatic disease than in non-cholestatic disease, although there was no significant difference identified. The reason for this is that the study did not include non-cirrhotic disease. The majority of patients with ESLD have a certain level of cholestasis. The current study demonstrated that cholestasis is a major risk factor for osteoporosis.

Significant differences between patients with and without osteoporosis were observed for age, female gender and total bile acid. Upon multivariate analysis, age and total bile acid were identified as risk factors for osteoporosis and predictive scores for osteoporosis were devised for patients with ESLD. If the score is ≤0.174, sensitivity is 0.815 and specificity is 0.62, patients require DEXA and treatment.

There were certain limitations of the present study. This was a retrospective study from a single hospital and had a small sample size. In future, a study of patients with ESLD from multiple centers is required to validate the current results.

In conclusion, age and total bile acid were identified as significant predictors of osteoporosis in patients with ESLD. In addition, the risk score calculated by multivariate analysis may be a useful marker for the prediction of osteoporosis in patients with ESLD.

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