World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.4254/wjh.v5.i11.627

World J Hepatol 2013 November 27; 5(11): 627-634
ISSN 1948-5182 (online)

BRIEF ARTICLE

Increased bone mineral density in patients with non-alcoholic steatohepatitis

Muhsin Kaya, Devran Işık, Remzi Beştaş, Osman Evliyaoğlu, Veysi Akpolat, Hüseyin Büyükbayram, Mehmet Ali Kaplan

Muhsin Kaya, Remzi Beştaş, Department of Gastroenterology, Dicle University, School of Medicine, Diyarbakır 21100, Turkey
Devran Işık, Mehmet Ali Kaplan, Department of Internal Medicine, Dicle University, School of Medicine, Diyarbakır 21100, Turkey
Osman Evliyaoğlu, Department of Biochemistry, Dicle University, School of Medicine, Diyarbakır 21100, Turkey
Veysi Akpolat, Department of Biophysics, Dicle University, School of Medicine, Diyarbakır 21100, Turkey
Hüseyin Büyükbayram, Department of Pathology, Dicle University, School of Medicine, Diyarbakır 21100, Turkey
Author contributions: Kaya M designed the study, performed all liver biopsies and wrote the manuscript; Kaya M, Kaplan MA, Işık D, Beştaş R collected data; Evliyaoğlu O performed all biochemical analyses; Akpolat V measured bone mineral density; Büyükbayram H evaluated liver biopsy specimens; and Kaplan MA performed the statistical analysis.
Correspondence to: Muhsin Kaya, MD, Department of Gastroenterology, Dicle University, School of Medicine, Diyarbakır 21100, Turkey. muhsinkaya20@hotmail.com
Telephone: +90-412-2488001 Fax: +90-412-2488002
Received: July 20, 2013 Revised: September 25, 2013
Accepted: October 11, 2013
Published online: November 27, 2013

Abstract

AIM: To determine the relationship between non-alcoholic steatohepatitis (NASH) and bone mineral density (BMD).

METHODS: A total of 38 patients (25 males) with a diagnosis of histologically proven NASH and 42 healthy controls (24 males) were enrolled in the study. Demographic features, clinical findings, complete blood count and routine biochemical analysis, as well as adrenal, thyroid and gonadal functions, were recorded. Additionally, intact parathormone, 25-OH-vitamin-D3, tumor necrosis factor-α, interleukin-6, interleukin-1, insulin-like growth factor-1 and insulin-like growth factor binding protein-3 levels were measured in both groups. Furthermore, lumbar spine and femoral neck BMD of both groups were measured by the dual-energy X-ray absorptiometry (DXA) method.

RESULTS: The mean age was 41 ± 12 years in the NASH group and 43 ± 11 years in the control group. Among demographic features, waist circumference was significantly larger in the NASH group compared to the control group ($P < 0.019$). Among laboratory parameters, serum triglyceride ($P < 0.008$), alanine transaminase ($P < 0.0001$), aspartate transaminase ($P < 0.001$), alkaline phosphatase ($P < 0.016$), gamma glutamyl transferase ($P < 0.0001$), ferritin ($P < 0.001$) and 25-OH-vitamin-D3 levels ($P < 0.0001$) were significantly higher in the NASH group compared to the control group. Lumbar BMD was significantly higher in the NASH group compared to the control group (1.057 ± 0.119 g/cm^2 *vs* 0.941 ± 0.133 g/cm^2; $P < 0.001$, respectively). In the NASH group, there was no significant relationship between BMD and fibrosis stage in liver biopsy.

CONCLUSION: NASH increases BMD and may be related to an elevated serum 25-OH-vitamin D3 level.

Key words: Non-alcoholic steatohepatitis; Hepatic osteodystrophy; Bone mineral density

Core tip: Identifying the relationship between non-alcoholic steatohepatitis and bone mineral density (BMD) and its underlying mechanism is important. We found that patients with biopsy-proven non-alcoholic steatohepatitis (NASH) had higher lumbar BMD and serum 25-OH-vitamin-D3 levels compared to healthy controls. We did not find a significant relationship between serum levels of thyroid hormones, sex hor-

mones, parathormone and cytokines, such as tumor necrosis factor-α, interleukin-1 (IL-1), IL-6, insulin-like growth factor-1, IGFBP-3 levels and BMD. An elevated serum 25-OH-vitamin D3 level may be the principle responsible factor in the increased bone mineral density in patients with NASH.

Kaya M, Işık D, Beştaş R, Evliyaoğlu O, Akpolat V, Büyükbayram H, Kaplan MA. Increased bone mineral density in patients with non-alcoholic steatohepatitis. *World J Hepatol* 2013; 5(11): 627-634 Available from: URL: http://www.wjgnet.com/1948-5182/full/v5/i11/627.htm DOI: http://dx.doi.org/10.4254/wjh.v5.i11.627

INTRODUCTION

The histological spectrum of non-alcoholic fatty liver disease (NAFLD) spans from generally benign, bland steatosis to steatosis with evidence of hepatocellular inflammation and damage (non-alcoholic steatohepatitis or NASH), which may be complicated by progressive fibrosis and cirrhosis[1,2]. Insulin resistance, oxidative stress and an inflammatory cascade are believed to play integral roles in the pathogenesis and progression of NAFLD[3]. Tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) induce insulin resistance by inhibiting the activation of insulin receptor substrate[4]. Low serum insulin-like growth factor-1 (IGF-1) levels are associated with a determinant of the metabolic syndrome, including insulin resistance, serum leptin levels, waist-to-hip ratio and type 2 diabetes mellitus[5]. In the circulating blood, most of the IGF-1 binds serum insulin-like growth factor binding protein-3 (IGFBP-3), which therefore lowers the bioavailability of IGF-1[6].

Hepatic osteodystrophy is a bone disease of multifactorial origin associated with chronic liver disease[7]. Osteoporosis accounts for the majority of cases, whereas osteomalacia is rare in the absence of advanced liver disease and severe malabsorption. The reported prevalence of osteoporosis among patients with chronic liver disease ranges from 20% to 100%, depending on patient selection and diagnostic criteria[8]. The pathogenesis is considered as multifactorial and remains unclear in some aspects[9]. Histologically, hepatic osteodystrophy is similar to postmenopausal and aging-related bone loss in that trabecular bone is more rapidly and severely affected than cortical bone[8]. Decreased bone mineral density (BMD) has been reported in patients with primary biliary cirrhosis, primary sclerosing cholangitis, chronic active hepatitis and patients with alcoholic liver disease[9]. Recently, decreased BMD in obese children with NASH[10,11] and in female patients with ultrasoud-proven NASH[12] have been reported.

The aims of our study were to determine: (1) BMD in patients with biopsy proven NASH; (2) the relationship between BMD and clinical parameters; and (3) the relationship between BMD and laboratory parameters.

MATERIALS AND METHODS

Patient population

A total of 38 patients with a diagnosis of NASH were prospectively and consecutively enrolled in the study. The diagnosis of NASH was unequivocally established in all patients based on the following criteria: (1) persistently raised ALT level (> 1.5 times the upper normal limit) for more than 6 mo; (2) a liver biopsy showing the presence of steatosis (> 5%), as well as lobular and/or portal inflammation, with or without Mallory bodies, fibrosis or cirrhosis; and (3) appropriate exclusion of other liver disease, such as alcoholic liver disease, viral hepatitis, autoimmune hepatitis, drug or toxin induced liver disease, primary biliary cirrhosis, biliary obstruction, space occupying lesions in the liver, hemochromatosis, Wilson's disease and α-1 antitrypsin-deficiency-associated liver disease.

All patients had a history of no alcohol consumption, confirmed by family members who were in close contact with the patients. No patient had a history of gastrointestinal surgery or ingestion of drugs known to produce hepatic steatosis in the previous 6 mo. None of the patients had been treated with drugs for the treatment of NASH before liver biopsy. Clinical symptoms and physical examination findings were recorded in all patients. The presence and absence of a space occupying lesion was verified by ultrasonography. At the time of the study, none of the patients showed clinical, biochemical or histological evidence of cirrhosis. In all cases, liver biopsies were performed as part of the evaluation of abnormal liver biochemistry.

Control group

Fifty healthy subjects without risk factors for laboratory and ultrasonographic evidence of liver disease were included in the study. All cases were selected from people who applied to our hospital for a routine check-up and who had no complaints. No cases had a history of alcohol intake and drug use. In all cases, complete physical examination and abdominal ultrasonography were performed. Cases with steatosis on ultrasonography and elevated liver enzymes were excluded. Of the 50 cases originally included in the study, 5 were excluded due to the presence of steatosis on ultrasonography and 3 were excluded from the study because of elevated liver enzymes. Liver biopsy was not performed in the control group.

Clinical and laboratory measurements

Body mass index (BMI) was calculated using the following formula: weight (kg)/height (m^2). We defined obesity as a BMI greater than 30. Subjects fasted overnight before blood samples were obtained. The index of insulin resistance was calculated using the fasting value of plasma glucose and the serum level, according to the homeostasis model assessment (HOMA) index as [(insulin) × (glucose)]/22.5. Abdominal ultrasonographic exami-

Table 1 Demographic features of patients in the non-alcoholic steatohepatitis and in the control group

Parameter	NASH group mean ± SD (range)	Control group mean ± SD (range)	*P* value
n (Female/Male)	13/25	18/24	0.428
Age (yr)	41 ± 12 (18-69)	42.8 ± 10 (24-65)	0.499
Smoking *n* (%)	10 (26)	9 (26)	0.810
BMI (kg/m²)	29 ± 4 (20-39)	27 ± 5 (21-39)	0.338
Waist (cm)	98 ± 9 (77-118)	93 ± 9 (76-113)	0.019

NASH: Non-alcoholic steatohepatitis; BMI: Body mass index.

nations were performed using 7.5 MHz probe (Toshiba SSH-140 A machine).

In all subjects, complete blood count, glucose, urea, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin, albumin, cholesterol, triglyceride, hepatitis B surface antigen, antibody to hepatitis B surface antigen, antibody to hepatitis C virus, antinuclear antibody, anti-smooth muscle antibody, anti-mitochondrial antibody, ferritin, ceruloplasmin, α-1 antitrypsin, insulin, intact parathormone (iPTH), 25-OH-vitamin D3, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), testosterone, free T4 (FT4), thyroid stimulating hormone (TSH) and dehydroepiandrostenedione sulfate (DHEAS) were determined by standard laboratory techniques. Because of seasonal variations in serum vitamin D levels[13], the control group and NASH group were studied in the same season. Measurement of IGF-1, IGFBP-3, TNF-α, IL-1 and IL-6 were performed by chemiluminescent immunometric assay on an automated system (Immulite 1000, Diagnostic Products Corp., Los Angeles, CA, United States).

Liver histology

A liver biopsy was performed in all patients and stained with hematoxylin-eosin, Masson's trichrome and rhodanine. Each liver biopsy was evaluated by an experienced pathologist to determine the severity of liver injury, steatosis, inflammatory cell accumulation, necroinflammatory activity and fibrosis. The degree of fibrosis was assessed using a 5 grade scale: 0 = none, normal connective tissue; 1 = mild, foci of pericellular fibrosis in zone 3; 2 = moderate, perivenular or pericellular fibrosis confined to zone 3 and 2 regions, with or without portal/periportal fibrosis; 3 = severe, bridging or septal fibrosis; and 4 = cirrhosis. The level of fatty infiltration was assessed and graded on a scale of 1 to 3: 1 = mild (5%-33% of hepatocytes affected); 2 = moderate (33%-66%); 3 = severe (> 66%). Lobular inflammation was assessed semi-quantitatively following the criteria of Brunt *et al*[14]. The presence or absence of Mallory bodies was recorded in all liver biopsies.

BMD measurement

BMD was measured by the dual-energy X-ray absorptiometry (DXA) method using Hologic machines (Hologic Discovery QDR 4500A, Waltham United States). Bone mass was expressed in absolute values (g/cm^2), T-score (number of standard deviations compared with a young (30-year-old) adult sex-matched reference population) and Z-score (number of standard deviations compared with an age and sex-matched reference population). As defined by World Health Organization, a T-score between -1 and -2.5 indicates osteopenia, whereas a T-score less than -2.5 indicates osteoporosis (World Health Organization. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Technical report no. 843. Geneva, Switzerland: World Health Organization, 1994). A Z-score less than -2 indicates a value in the lowest 2.5th percentile of the reference range, a value associated with a considerably larger increase in the risk of fracture. The BMD of the lumbar spine at L_1-L_4 and BMD of the left femoral neck were measured in all patients in the NASH and the control group.

Statistical analysis

Results are expressed as mean ± SD and number of patients with a condition. Statistical analyses were carried out by using the statistical packages for SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, United States). Mean and SD were calculated for continuous variables. Differences between the groups according to non-numerical values were tested by the χ^2 test and Fisher exact tests. The normal distribution of numerical values was analyzed by the Kolmogorov-smirnov test. Normal distributed values were analyzed by the Student's *t*-test and non-normal distributed values were analyzed by the Mann-Whitney *U* test. Two-sided *P* values were considered statistically significant at $P \leqslant 0.05$. The study was approved by the Institutional Review Board and all patients gave informed consent for participation in this research.

RESULTS

NASH group

Table 1 shows the demographic features of both groups. There were 25 (60%) males and 13 (40%) females in the NASH group. In the clinical history, 22 (58%) patients had fatigue, 27 (71%) dyspepsia and 6 (16%) patients had right upper quadrant pain. Physical examination revealed obesity in 10 (26%), hypertension in 7 (18%) and hepatomegaly in 7 (18%) patients. None of the patients had space occupying lesions on the ultrasonographic examination.

Complete blood count was within normal range in all patients. Table 2 shows the biochemical findings of both groups. There were elevations in serum ALT levels in all (100%), AST in 34 (89%), alkaline phosphatase in 4 (11%), GGT in 21 (55%), total bilirubin in 5 (13%), total cholesterol in 12 (32%), triglyceride in 22 (58%) and ferritin in 15 (39%) patients. Serum urea, creatinine, calcium and phosphorus levels were within normal limits in all patients. Two patients had slightly decreased serum

Table 2 Biochemical findings of the non-alcoholic steatohepatitis and the control group

Parameter	NASH group mean ± SD (range)	Control group mean ± SD (range)	*P* value
Urea (mg/dL)	28 ± 7 (13-52)	29 ± 8 (14-51)	0.631
Creatinine (mg/dL)	0.7 ± 0.1 (0.6-1.1)	0.8 ± 0.1 (0.5-1.1)	0.631
T. cholesterol (mg/dL)	191 ± 42 (104-314)	187 ± 44 (98-296)	0.668
Triglyceride (mg/dL)	208 ± 210 (67-1420)	136 ± 67 (33-298)	0.008
Calcium (mg/dL)	9.5 ± 0.4 (8.6-10.3)	9.4 ± 0.54 (8.6-10.6)	0.239
Phosphorus (mg/dL)	3.4 ± 0.4 (2.1-4.3)	3.6 ± 0.6(2.3-5.7)	0.288
ALT (U/L)	114 ± 115 (51-775)	20 ± 8 (8-36)	< 0.0001
AST (U/L)	55 ± 31 (28-190)	20 ± 7 (2-35)	< 0.0001
ALP (U/L)	93 ± 44 (43-266)	72 ± 18 (44-123)	0.016
GGT (U/L)	121 ± 214 (23-1135)	29 ± 15 (8-65)	< 0.0001
T bilirubin (mg/dL)	0.8 ± 0.6 (0.2-3.2)	0.6 ± 0.3 (0.2-1.5)	0.165
Albumin (g/dL)	4.2 ± 0.5 (2.3-5.1)	4.2 ± 0.3 (3.5-5.1)	0.214
Ferritin (ng/mL)	208 ± 193 (22-901)	75 ± 82 (3-470)	< 0.001

NASH: Non-alcoholic steatohepatitis; AST: Aspartate transaminase; ALT: Alanine transaminase; GGT: Gamma glutamyl transferase; ALP: Alkaline phosphatase.

Table 3 Serum hormone and cytokine results of the non-alcoholic steatohepatitis and the control group

Parameter	NASH group mean ± SD (range)	Control group mean ± SD (range)	*P* value
HOMA	5.7 ± 5.25 (1.1-23.59)	5.6 ± 10.1 (0.7-59.9)	0.192
Cortisol (μg/dL)	12.6 ± 5.8 (1.6-27)	11.6 ± 5.8 (2.9-28)	0.375
TSH (uIU/mL)	1.7 ± 1.09 (0.2-5.3)	1.36 ± 1 (0.01-4)	0.104
FT4 (ng/dL)	1.22 ± 0.19 (0.9-1.8)	1.22 ± 0.22 (0.8-107)	0.999
FSH (mIU/mL)	21 ± 41 (1.6-299)	19 ± 37 (0.3-177)	0.729
LH (mIU/mL)	10.4 ± 10.8 (1.2-44)	11 ± 15 (0.1-64)	0.974
E2 (pg/mL)	39.1 ± 72 (3.8-435)	41 ± 47 (2-199)	0.404
DHEAS (ug/dL)	190 ± 109 (25-501)	146 ± 90 (33-366)	0.071
iPTH (pg/mL)	46.3 ± 20 (18-130)	50 ± 16 (19-105)	0.086
25-OH-vit-D3 (ug/L)	17 ± 6 (5.1-39)	13 ± 7 (3.8-36)	< 0.0001
Testosterone (ng/mL)	2.5 ± 1.9 (0.03-5.8)	1.7 ± 1.9 (0.02-5.4)	0.075
IGF-1 (ng/mL)	141 ± 94 (30-527)	144 ± 47 (67-292)	0.099
TNF-α (pg/mL)	11.6 ± 6.7 (4-31.7)	14 ± 7 (2-4.9)	0.062
IL-6 (pg/mL)	2.2 ± 0.6 (2-4.9)	3 ± 2.4 (2-12)	0.8
IL-1 (pg/mL)	8.2 ± 16.5 (5-107)	5.3 ± 2 (5-17)	0.291
IGFBP-3 (ug/mL)	4.1 ± 1.68 (1.1-8.6)	4.2 ± 0.8 (2.6-6.02)	0.909

NASH: Non-alcoholic steatohepatitis; TNF-α: Tumor necrosis factor-α; IL-6: Interleukin-6; IGF-1: Insulin-like growth factor-1; IGFBP-3: Insulin-like growth factor binding protein-3; HOMA: Homeostasis model assessment; TSH: Thyroid stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; DHEAS: Dehydroepiandrostenedione sulfate; iPTH: Intact parathormone; FT4: Free T4; FSH: Follicle-stimulating hormone.

albumin levels.

Table 3 shows hormone and cytokine levels of both groups. There were elevations in the serum cortisol level in 3 (8%) patients, TSH in 1 (3%) and iPTH level in 4 (11%) patients. Serum FSH, LH, DHEAS and testosterone levels were within normal range in all patients. We found decreased serum 25-OH-vitamin D3 levels in 4 (11%) patients and decreased serum IGF-1 levels in 16 (42%) patients. There were elevations in the serum TNF-α level in 16 (42%), IL-1 in 2 (5%) and IGFBP-3 in 16 (42%) patients. Serum IL-6 level was within normal range in all patients.

Table 4 shows histopathological analysis of the liver section obtained from patients in the NASH group. Most of the patients (71%) had moderate or severe steatosis. There was mild or moderate fibrosis (grade I or Ⅱ) in 21 (55%) patients. Twelve patients (32%) had no fibrosis and none of the patients had cirrhosis in the liver biopsy.

Table 5 shows the bone mineral density of both groups. Five male patients (11%) had osteoporosis, 13 (34%) patients (9 males and 2 premenopausal and 2 postmenopausal females) had osteopenia and 20 (57%) patients had normal bone mineral density.

Control group

There were 18 (42.8%) females and 24 (57%) males in the control group. Physical examination revealed hypertension in 10 (23.8%) and obesity in 13 (30.9%) patients.

Complete blood count, serum urea, creatinine, ALT, AST, alkaline phosphatase and albumin levels were within normal range in all patients. There were elevations in serum levels of total cholesterol level in 13 (30.9%), triglyceride in 7 (16.6%), calcium in 3 (7.1%), phosphorus

Table 4 Histopathological findings according to Brunt classification of liver biopsy obtained from the non-alcoholic steatohepatitis group *n* (%)

Parameters	Grade 0	Grade 1	Grade 2	Grade 3
Steatosis	0 (0)	11 (29)	15 (39)	12 (32)
Ballooning	1 (3)	11 (29)	26 (68)	-
Lobular inflammation	0 (0)	21 (55)	16 (42)	1 (3)
Portal inflammation	8 (21)	24 (63)	6 (16)	0 (0)
Fibrosis	12 (32)	18 (47)	3 (8)	5 (13)

in 1 (2.3%), GGT in 3 (7.1%), total bilirubin in 1 (2.3%) and ferritin in 3 (7.1%) patients.

There were elevations in the serum levels of cortisol in 4 (9.5 11%), THS in 1 (2.3%) and iPTH in 6 (14.2%) patients. Decreased serum 25-OH-vitamin D3 levels were found in 14 (33.3%) patients. Serum FSH, LH, E2, testosterone, DHEAS and FT4 levels were within normal range in all patients.

There were elevations in the serum levels of TNF-α in 23 (54.7%), IL-1 in 1 (2.3%), IL-6 in 1 (2.3%) and IGFBP-3 in 15 (35.7%) patients. Decreased serum IGF-1 levels were found in only 13 (30.9%) patients.

There was osteoporosis in 11 (26.1%) (5 males, 3 premenopausal and 3 postmenopausal females) patients. There was osteopenia in 13 (30.9%) (7 males, 5 premenopausal and 1 postmenopausal females) patients. Bone mineral density was within normal range in 11 (26.1%) patients.

Comparison of the NASH and the control group

There were no significant differences between the NASH group and control group regarding age, gender, BMI and the incidence of smoking and hypertension. Waist circumference was significantly larger in the NASH group compared to the control group (98 ± 9 cm *vs* 93 ± 9 cm, respectively; $P = 0.019$). There were no significant differences between the NASH group and the control group regarding complete blood count, HOMA score, serum urea, creatinine, total cholesterol, calcium, phosphorus, total bilirubin, albumin, cortisol, TSH, FT4, FSH, LH, E2, DHEAS, iPTH, IGF-1, TNF-α, IL-1, IL-6 and IGFBP-3 levels.

Serum triglyceride (208 ± 210 *vs* 136 ± 67; $P = 0.008$), ALT (114 ± 115 *vs* 20 ± 8; $P < 0.0001$), AST (55 ± 31 *vs* 20 ± 7; $P < 0.001$), ALP (93 ± 44 *vs* 72 ± 18; $P = 0.016$), GGT (121 ± 214 *vs* 29 ± 15; $P < 0.0001$), ferritin (208 ± 193 *vs* 75 ± 82; $P < 0.001$) and 25-OH-vitamin D3 levels (17 ± 6 *vs* 13 ± 7; $P < 0.0001$) were significantly higher in the NASH group compared to the control group.

Lumbar BMD was significantly higher in the NASH group compared to the control group (1.057 ± 0.119 g/cm^2 *vs* 0.941 ± 0.133 g/cm^2, $P = 0.001$). Lumbar T-score and Z-score were also significantly higher in the NASH group compared to the control group [(-0.77) ± (1.25) *vs* (-1.58) ± (1.36); $P = 0.009$ and (-0.44) ± (1.38) *vs* (-1.18) ± (1.28); $P = 0.019$; respectively]. There was no significant difference between the NASH group and control group regarding femoral BMD, femoral T-score and Z-score.

DISCUSSION

Hepatic osteodystrophy is a bone disease of multifactorial origin associated with chronic liver disease. Both osteoporosis and osteopenia are part of this condition. Histologically, hepatic osteodystrophy is similar to postmenopausal and aging-related bone loss in that trabecular bone is more rapidly and severely affected than cortical bone[8]. On the basis of BMD measurements, the reported prevalence of low BMD ranges from 13 to 70%[8,15]. Cholestatic liver disease has a higher incidence of hepatic osteodystrophy than non cholestatic liver disease, but BMD loss is present in cirrhosis of all etiologies. The mean T-score evaluated by DXA in the lumbar spine has been found to be -2.22 in primary biliary cirrhosis, -1.93 in primary sclerosing cholangitis, -1.23 in chronic active hepatitis and -0.86 in alcoholic cirrhosis[9]. Decreased BMD in obese children with NASH[10,11] and in female patients with ultrasound-proven steatosis and elevated ALT[12] have been reported. Our findings were not compatible with previous studies. We found that NASH has a promoting effect on lumbar BMD compared to healthy controls (-1.057 ± 0.119 g/cm^2 *vs* 0.941 ± 0.133 g/cm^2, $P = 0.001$ respectively). Our study population consisted of an adult population with both genders and most patients with NASH had low fibrosis scores on liver biopsy. We also found that patients with NASH had a higher level of serum 25-OH-vitamin-D3 compared to healthy controls. NASH has an insignificant promoting effect on femoral BMD compared to healthy controls. This different promoting effect on lumbar and femoral bone may be related to the structure of those bones.

Potential inciting factors that either directly or indirectly alter the bone mass include IGF-1 deficiency, hyperbilirubinemia, hypogonadism (estrogen and testosterone deficiency), alcoholism, excess tissue iron deposition, subnormal vitamin D levels, vitamin D receptor genotype, osteoprotegerin deficiency, vitamin K deficiency, immunosuppressive therapy preceding and following liver transplantation[9,10], together with less exercise and muscle activity compared to healthy persons[16]. Vitamin D is a key regulator of bone metabolism and several studies in adults have shown that vitamin D increases bone mineral density[17] and prevents osteoporotic fractures[18]. Among NAFLD, a significant correlation between decreased serum 25-hydroxyvitamin D concentration and histological severity of hepatic steatosis, necroinflammation and fibrosis have been reported previously[19,20]. There are inverse correlations between serum 25-OH-vitamin D3 levels and all adiposity measurement, including BMI percentage, waist circumference, total fat mass, percentage of body fat and subcutaneous abdominal adipose tissue[13,21]. There are seasonal variations in serum vitamin D levels and its level is higher in summer than winter[13]. There were no

Table 5 Dual-energy X-ray absorptiometry results of patients in the non-alcoholic steatohepatitis and the control group

Parameter	NASH group mean ± SD (range)	Control group mean ± SD (range)	*P* value
Lumbar BMD	1.057 ± 0.119	0.941 ± 0.133	0.001
Lumbar T-score	-0.77 ± 1.25 (-2.9-2.6)	-1.58 ± 1.36 (-3.7-2.2)	0.009
Lumbar Z-score	-0.44 ± 1.38 (-3.3-2.9)	-1.18 ± 1.28 (-2.9-2.5)	0.019
Femoral BMD	1.004 ± 0.118	0.972 ± 0.130	0.305
Femoral T-score	0.32 ± 0.87 (-2-1.8)	0.062 ± 1.02 (-1.7-2.1)	0.242
Femoral Z-score	0.65 ± 0.92 (-1.5-2.8)	0.35 ± 0.94 (-1.6-2)	0.178

NASH: Non-alcoholic steatohepatitis; BMD: Bone mineral density.

known factors that can cause hepatic osteodystrophy in our patients with NASH. In this study, there was an elevated serum 25-OH-vitamin-D3 level in the NASH group compared to the control group (17.3 ± 6.1 *vs* 14.07 ± 10.8; $P < 0.0001$, respectively). It may be the main responsible factor for the elevated BMD of patients with NASH. All our patients in the NASH group and control group were included in the study at the same season. Both serum 25-OH-vitamin D3 levels and mean waist circumferences were significantly higher in the NASH group compared to the control group. Therefore, our results were not compatible with previously reported literature. We suggest that the relationship between serum 25-OH-vitamin D3 levels and adiposity parameters, BMD and histopathological changes in NASH should be investigated in a multicenter, multiregional, prospective study.

Dehydroepiandrosterone (DHEA) and its sulfate derivative (DHEAS) are the most abundant circulating C_{19} steroids in humans and are produced primarily from the adrenal glands. The actions of DHEA in humans are thought to be mediated primarily through conversion to sex hormones[22]. DHEA is the precursor for 30%-50% of circulating androgens in older men[23] and more than 70% in older women[24]. It has been postulated that the decline in DHEA with aging contributes to physiological changes that are dependent on sex hormones, such as the loss of bone and muscle mass. It has been reported that DHEA replacement therapy for 1 year improved hip bone mineral density in older adults and spine bone mineral density in older women[25]. Charlton *et al*[26] reported that more advanced NAFLD is strongly associated with low circulating DHEAS. In our study, most of the patients with NASH had early grade (less than grade Ⅱ) liver fibrosis, verified by biopsy. Therefore, early stages of NASH may not have any negative effect on serum DHEAS levels. A high serum parathormone level has been associated with increased bone remodeling, excessive bone loss and increased fracture risk[27]. The inverse correlation between serum parathormone levels and BMD in all skeletal sites has been reported previously[28]. Although there was no statistically significant difference between the two groups, elevated serum DHEAS (190 ± 109 *vs* 146 ± 90; $P = 0.071$, respectively) and decreased serum iPTH levels (46.3 ± 20 *vs* 50 ± 16, $P = 0.086$, respectively) may have an additional promoting effect on the elevated bone mineral density in our patients with steatohepatitis. The concomitant elevation in serum 25-OH-vitamin D3 and DHEAS levels and decline in serum iPTH levels in the NASH group compared to the control group can explain pathophysiologically elevated BMD in our patients with NASH.

There is increasing evidence that several cytokines regulate metabolism, inflammatory response, cell death, regeneration and fibrosis in normal and injured liver tissue[29]. Obesity is characterized by a broad inflammatory response and many inflammatory mediators exhibit patterns of expression and/or impact insulin action that correlates with the progression of metabolic syndrome[30]. The tissue expression and circulating cytokines, such as TNF-α, IL-1, IL-6, adiponectin and interferon-γ, have been associated with obesity-related insulin resistance[31]. IGF-1 is a multipotent anabolic hormone with beneficial effects on glucose homeostasis by its action as an insulin sensitizing mediator. Hepatocytes are the main source of circulating IGF-1 whose secretion is stimulated by growth hormone[32]. Garcia-Galiano *et al*[33] reported glucose > 110 mg/dL, IL-6 > 4.81 pg/mL, IGF-1 < 130 ng/mL, HOMA > 4.5 and IGF-1 < 110 ng/mL as independent predictors of hepatic steatosis and NASH, respectively. In a population based study, positive correlation between hyperechogenic liver pattern and low serum IGF-1 and low serum IGF-1/IGFBP-3 ratios have been reported[34]. The relationship between low serum IGF-1 and fibrosis stage in patients with NAFLD has also been reported[35]. In our study, we did not find a significant relationship between serum TNF-α, IL-1, IL-6, IGF-1, IGFBP-3 and NASH. The prospective collection of all data, the presence of a control group and the histopathological diagnosis of NASH may enhance the validity of our study.

In conclusion, we found that NASH has a promoting effect on bone mineral density. This increase was not related to serum cytokine levels, including TNF-α, IL-1, IL-6, IGF-1 and IGFBP-3. Elevated serum 25-OH-vitamin D3 levels may be the main responsible factor for increased bone mineral density in NASH. Elevated serum DHEAS and decreased serum iPTH levels (but the significance level was not achieved) may have an additional promoting effect on bone mineral density in NASH.

COMMENTS

Background

Non-alcoholic steatohepatitis (NASH) may be complicated by progressive fibrosis and cirrhosis. Hepatic osteodystrophy is a bone disease of multifactorial origin associated with chronic liver disease and histologically similar to postmenopausal or aging-related bone loss. The pathogenesis is considered multifactorial and remains unclear in some aspects. The relationship between NASH and bone mineral density (BMD) is important topic.

Research frontiers

Potential inciting factors, such as the stage of liver disease, insulin-like growth factor 1 (IGF-1) levels, sex hormones, vitamin D, parathormone and circulating cytokine levels, that may influence BMD in NASH may give a new frontier to fight two prevalent conditions like NASH and osteoporosis.

Innovations and breakthroughs

Cholestatic liver disease has higher incidence of hepatic osteodystrophy than non cholestatic liver disease, but BMD loss is present in all etiologies of cirrhosis. There are a few publications regarding decreased BMD and its underlying mechanism in patients with NASH. In this study, the authors showed that patients with liver biopsy-proven NASH had a significantly higher lumbar BMD and 25-OH-vitamin-D3 level than the healthy control group. There was no significant relationship between BMD and fibrosis stage in liver biopsy of patients with NASH. This increase in lumbar BMD was also not related to serum TNF-α, interleukin (IL)-1, IL-6, IGF-1 and IGFBP-3. Elevated serum 25-OH-vitamin D3 level may be the main responsible factor for increased bone mineral density in NASH. The results of this study were not compatible with previously reported studies.

Applications

Elevated serum 25-OH-vitamin-D3 level may have a protective effect on BMD in patients with NASH. Therefore, supplemental supportive administration of 25-OH-vitamin-D3 to prevent bone loss in patients with NASH may not be required until the development of cirrhosis.

Terminology

NASH is steatosis with evidence of hepatocellular inflammation and damage of liver and it may progress to advanced fibrosis, cirrhosis and hepatocellular cancer. Hepatic osteodystrophy is a bone disease of multifactorial origin associated with chronic liver disease.

Peer review

Interestingly, the authors report that NASH patients have a higher lumbar BMD and 25-OH-vitamin D3 level than healthy controls.

REFERENCES

1 **Adams LA**, Angulo P, Lindor KD. Nonalcoholic fatty liver disease. *CMAJ* 2005; **172**: 899-905 [PMID: 15795412 DOI: 10.1503/cmaj.045232]

2 **Cheung O**, Sanyal AJ. Recent advances in nonalcoholic fatty liver disease. *Curr Opin Gastroenterol* 2009; **25**: 230-237 [PMID: 19396962 DOI: 10.1097/MOG.0b013e3283294a18]

3 **Lewis JR**, Mohanty SR. Nonalcoholic fatty liver disease: a review and update. *Dig Dis Sci* 2010; **55**: 560-578 [PMID: 20101463 DOI: 10.1007/s10620-009-1081-0]

4 **Hotamisligil GS**, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α- and obesity-induced insulin resistance. *Science* 1996; **271**: 665-668 [PMID: 8571133 DOI: 10.1126/science.271.5249.665]

5 **Gómez JM**, Maravall FJ, Gómez N, Navarro MA, Casamitjana R, Soler J. Interactions between serum leptin, the insulin-like growth factor-I system, and sex, age, anthropometric and body composition variables in a healthy population randomly selected. *Clin Endocrinol* (Oxf) 2003; **58**: 213-219 [PMID: 12580938 DOI: 10.1046/j.1365-2265.2003.01698.x]

6 **Yakar S**, Liu JL, Stannard B, Butler A, Accili D, Sauer B, LeRoith D. Normal growth and development in the absence of hepatic insulin-like growth factor I. *Proc Natl Acad Sci USA* 1999; **96**: 7324-7329 [PMID: 10377413]

7 **Choudhary NS**, Tomar M, Chawla YK, Bhadada SK, Khandelwal N, Dhiman RK, Duseja A, Bhansali A. Hepatic osteodystrophy is common in patients with noncholestatic liver disease. *Dig Dis Sci* 2011; **56**: 3323-3327 [PMID: 21573732 DOI: 10.1007/s10620-011-1722-y]

8 **Rouillard S**, Lane NE. Hepatic osteodystrophy. *Hepatology* 2001; **33**: 301-307 [PMID: 11124849]

9 **Gasser RW**. Cholestasis and metabolic bone disease - a clinical review. *Wien Med Wochenschr* 2008; **158**: 553-557 [PMID: 18998071 DOI: 10.1007/s10354-008-0594-z]

10 **Pardee PE**, Dunn W, Schwimmer JB. Non-alcoholic fatty liver disease is associated with low bone mineral density in obese children. *Aliment Pharmacol Ther* 2012; **35**: 248-254 [PMID:22111971 DOI: 10.1111/j.1365-2036.2011.04924.x]

11 **Pacifico L**, Bezzi M, Lombardo CV, Romaggioli S, Ferraro F, Bascetta S, Chiesa C. Adipokines and C-reactive protein in relation to bone mineralization in pediatric nonalcoholic fatty liver disease. *World J Gastroenterol* 2013; **19**: 4007-4014 [PMID: 23840146 DOI: 10.3748/wjg.v19.i25.4007]

12 **Purnak T**, Beyazit Y, Ozaslan E, Efe C, Hayretci M. The evaluation of bone mineral density in patients with nonalcoholic fatty liver disease. *Wien Klin Wochenschr* 2012; **124**: 526-531 [PMID: 22850810]

13 **Dong Y**, Pollock N, Stallmann-Jorgensen IS, Gutin B, Lan L, Chen TC, Keeton D, Petty K, Holick MF, Zhu H. Low 25-hydroxyvitamin D levels in adolescents: race, season, adiposity, physical activity, and fitness. *Pediatrics* 2010; **125**: 1104-1111 [PMID: 20439594 DOI: 10.1542/peds.2009-2055]

14 **Brunt EM**, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474 [PMID: 10484010 DOI: 10.1111/j.1572-0241.1999.01377.x]

15 **Carey EJ**, Balan V, Kremers WK, Hay JE. Osteopenia and osteoporosis in patients with end-stage liver disease caused by hepatitis C and alcoholic liver disease: not just a cholestatic problem. *Liver Transpl* 2003; **9**: 1166-1173 [PMID: 14586877 DOI: 10.1053/jlts.2003.50242]

16 **George J**, Ganesh HK, Acharya S, Bandgar TR, Shivane V, Karvat A, Bhatia SJ, Shah S, Menon PS, Shah N. Bone mineral density and disorders of mineral metabolism in chronic liver disease. *World J Gastroenterol* 2009; **15**: 3516-3522 [PMID: 19630107 DOI: 10.3748/wjg.15.3516]

17 **Bischoff-Ferrari HA**, Dietrich T, Orav EJ, Dawson-Hughes B. Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. *Am J Med* 2004; **116**: 634-639 [PMID: 15093761 DOI: 10.1016/j.amjmed.2003.12.029]

18 **Bischoff-Ferrari HA**, Willett WC, Wong JB, Giovannucci E, Dietrich T, Dawson-Hughes B. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. *JAMA* 2005; **293**: 2257-2264 [PMID: 15886381 DOI: 10.1001/jama.293.18.2257]

19 **Targher G**, Bertolini L, Scala L, Cigolini M, Zenari L, Falezza G, Arcaro G. Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis* 2007; **17**: 517-524 [PMID: 16928437 DOI: 10.1016/j.numecd.2006.04.002]

20 **Manco M**, Ciampalini P, Nobili V. Low levels of 25-hydroxyvitamin D(3) in children with biopsy-proven nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 2229; author reply 2230 [PMID: 20513013 DOI: 10.1002/hep.23724]

21 **Caron-Jobin M**, Morisset AS, Tremblay A, Huot C, Légaré D, Tchernof A. Elevated serum 25(OH)D concentrations, vitamin D, and calcium intakes are associated with reduced adipocyte size in women. *Obesity* (Silver Spring) 2011; **19**: 1335-1341 [PMID: 21527900 DOI: 10.1038/oby.2011.90]

22 **Orentreich N**, Brind JL, Rizer RL, Vogelman JH. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. *J Clin Endocrinol Metab* 1984; **59**: 551-555 [PMID: 6235241 DOI: 10.1210/

jcem-59-3-551]
23 **Labrie F**, Dupont A, Belanger A. Complete androgen blockade for the treatment of prostate cancer. *Important Adv Oncol* 1985; 193-217 [PMID: 3916740]
24 **Davison SL**, Bell R, Donath S, Montalto JG, Davis SR. Androgen levels in adult females: changes with age, menopause, and oophorectomy. *J Clin Endocrinol Metab* 2005; **90**: 3847-3853 [PMID: 15827095 DOI: 10.1210/jc.2005-0212]
25 **Jankowski CM**, Gozansky WS, Schwartz RS, Dahl DJ, Kittelson JM, Scott SM, Van Pelt RE, Kohrt WM. Effects of dehydroepiandrosterone replacement therapy on bone mineral density in older adults: a randomized, controlled trial. *J Clin Endocrinol Metab* 2006; **91**: 2986-2993 [PMID: 16735495 DOI: 10.1210/jc.2005-2484]
26 **Charlton M**, Angulo P, Chalasani N, Merriman R, Viker K, Charatcharoenwitthaya P, Sanderson S, Gawrieh S, Krishnan A, Lindor K. Low circulating levels of dehydroepiandrosterone in histologically advanced nonalcoholic fatty liver disease. *Hepatology* 2008; **47**: 484-492 [PMID: 18220286 DOI: 10.1002/hep.22063]
27 **Mosekilde L**. Primary hyperparathyroidism and the skeleton. *Clin Endocrinol* (Oxf) 2008; **69**: 1-19 [PMID: 18167138 DOI: 10.1111/j.1365-2265.2007.03162.x]
28 **Arabi A**, Baddoura R, El-Rassi R, El-Hajj Fuleihan G. PTH level but not 25 (OH) vitamin D level predicts bone loss rates in the elderly. *Osteoporos Int* 2012; **23**: 971-980 [PMID: 21656018 DOI: 10.1007/s00198-011-1659-1]
29 **Andus T**, Bauer J, Gerok W. Effects of cytokines on the liver. *Hepatology* 1991; **13**: 364-375 [PMID: 1995444]
30 **Wellen KE**, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest* 2005; **115**: 1111-1119 [PMID: 15864338 DOI: 10.1172/JCI200525102DS1]
31 **Kern PA**, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab* 2001; **280**: E745-E751 [PMID: 11287357]
32 **Schmid C**. Insulin-like growth factors. *Cell Biol Int* 1995; **19**: 445-457 [PMID: 7640658 DOI: 10.1006/cbir.1995.1088]
33 **García-Galiano D**, Sánchez-Garrido MA, Espejo I, Montero JL, Costán G, Marchal T, Membrives A, Gallardo-Valverde JM, Muñoz-Castañeda JR, Arévalo E, De la Mata M, Muntané J. IL-6 and IGF-1 are independent prognostic factors of liver steatosis and non-alcoholic steatohepatitis in morbidly obese patients. *Obes Surg* 2007; **17**: 493-503 [PMID: 17608262]
34 **Völzke H**, Nauck M, Rettig R, Dörr M, Higham C, Brabant G, Wallaschofski H. Association between hepatic steatosis and serum IGF1 and IGFBP-3 levels in a population-based sample. *Eur J Endocrinol* 2009; **161**: 705-713 [PMID: 19690083 DOI: 10.1530/EJE-09-0374]
35 **Ichikawa T**, Nakao K, Hamasaki K, Furukawa R, Tsuruta S, Ueda Y, Taura N, Shibata H, Fujimoto M, Toriyama K, Eguchi K. Role of growth hormone, insulin-like growth factor 1 and insulin-like growth factor-binding protein 3 in development of non-alcoholic fatty liver disease. *Hepatol Int* 2007; **1**: 287-294 [PMID: 19669352 DOI: 10.1007/s12072-007-9007-4]

P- Reviewers: Khedmat H, Kiemer AK, Ozenirler S
S- Editor: Wen LL **L- Editor**: Roemmele A **E- Editor**: Liu XM

百世登
Baishideng®

Published by **Baishideng Publishing Group Co., Limited**
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road, Wan Chai, Hong Kong, China
Fax: +852-65557188
Telephone: +852-31779906
E-mail: bpgoffce@wjgnet.com
http://www.wjgnet.com