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Bone Mineral Density, Bone Turnover, and Systemic Inflammation in Non-cirrhotics with Chronic Hepatitis C

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Abstract

Background—Whether chronic HCV, a disease characterized by systemic inflammation, impacts bone mineral density (BMD) *independent of cirrhosis* is unknown. *Aim* We aimed to evaluate the association between BMD, systemic inflammation, and markers of bone turnover in chronic HCV without cirrhosis.

Methods—Non-cirrhotics, 40–60 years old, with chronic HCV underwent measurement of: (1) BMD by dual-energy X-ray absorptiometry scan and (2) serum markers of systemic inflammation and bone turnover. By Chi-squared or *t* test, we compared those with normal versus low BMD.

Results—Of the 60 non-cirrhotics, 53 % were female and 53 % Caucasian. Mean (SD) age was 53.3 years (5.7), total bilirubin 0.7 mg/dL (0.3), creatinine 0.8 mg/dL (0.2), and body mass index 28.4 kg/m^2 (6.5). Low BMD was observed in 42 %: 30 % had osteopenia, 12 % had osteoporosis. Elevated tumor necrosis factor α, interleukin-6, and C-reactive protein levels were found in 26, 32,

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and 5 %, respectively, but did not differ by BMD group ($p > 0.05$). Patients with low BMD had higher serum phosphorus (4.1 vs. 3.5 mg/dL) and pro-peptide of type 1 collagen (P1NP; 73.1 vs. 47.5 ng/mL) [$p < 0.05$], but similar bone-specific alkaline phosphatase, serum C-telopeptide, and parathyroid hormone levels.

Conclusions—Low BMD is prevalent in 40- to 60-year-old non-cirrhotics with chronic HCV, but not associated with systemic inflammatory markers. Elevated P1NP levels may help to identify those at increased risk of bone complications in this population. Chronic HCV should be considered a risk factor for bone loss, prompting earlier BMD assessments in both men and women.

Keywords

Bone turnover; Inflammation; Osteoporosis; Hepatic osteodystrophy

Introduction

Over 4 million individuals in the USA are infected with chronic hepatitis C virus (HCV) [1]. Approximately 5–30 % of these individuals will progress to cirrhosis after 20 years of infection [2]. One well-recognized extrahepatic complication of cirrhosis is hepatic osteodystrophy, a term that encompasses the development of low bone mineral density in the setting of chronic liver disease. Studies have reported that up to 53 % of patients with cirrhosis have osteopenia or osteoporosis [3–6], putting them at risk of disabling and potentially life-threatening atraumatic fractures. In addition, HCV-related liver disease is currently the leading indication for liver transplantation in the USA [7], carrying a 20 % risk of fracture within the first year after transplantation [8, 9], suggesting that the pre-transplant environment plays a strong role in the risk of this post-transplant complication.

Although the pathogenesis of bone disease in patients with HCV-related cirrhosis is not completely understood, it is likely that a combination of traditional skeletal risk factors including hypogonadism [6] and vitamin D deficiency [10, 11] as well as low levels of insulin-like growth factor [3, 12] contributes to bone loss in the end-stages of chronic liver disease. However, one small study found that over 50 % of the 30 HCV-infected patients *without* evidence of cirrhosis had osteopenia or osteoporosis by dual-energy X-ray absorptiometry (DXA) despite adequate levels of 25(OH)vitamin D judged sufficient by these investigators (mean 27 ng/mL) [13]. This suggests that alternative mechanisms are needed to explain bone loss prior to the onset of cirrhosis. As chronic HCV infection involves persistent systemic inflammation [14, 15], it is possible that this pro-inflammatory state plays a critical role in the progression of bone disease.

Thus far, few studies have investigated bone mineral density (BMD) in HCV-infected patients, but the association between chronic inflammation and bone loss is well documented in other inflammatory states. Compared with healthy controls matched for menopausal status, patients with early rheumatoid arthritis who have not been exposed to corticosteroids or disease-modifying agents have significantly lower BMD. In these patients, disease activity, as measured by C-reactive protein (CRP), is a strong predictor of bone loss [16]. Osteoporosis, defined by a BMD *T* score <−2.5 by DXA scan, has been reported in up

to 28 % of patients with Crohn's disease [17]. Treatment with infliximab, an agent targeted against TNF-α, is associated with improved bone mass in the lumbar spine, suggesting that this pro-inflammatory cytokine is important in bone loss [18].

Even independent of specific diseases, inflammation, as measured by high-sensitivity CRP, has been shown to be an independent risk factor for non-traumatic fracture [19, 20]. Inflammation modulates the bone-remodeling pathway mainly by two mechanisms. First, pro-inflammatory cytokines, including TNF-α, interleukin (IL)-1, IL-6, IL-17, and macrophage colony-stimulating factor (M-CSF), induce expression of receptor activator of nuclear factor κβ ligand (RANK-L), thereby increasing differentiation of osteoclasts from their precursor cells [21, 22]. Second, TNF-α has been shown to play an additional role in this pathway by downregulating bone anabolic pathways, blunting osteoclastogenesis [23, 24]. Therefore, chronic inflammation disrupts the balance of activity of osteoclasts and osteoblasts and by favoring bone resorption, precipitates bone loss.

The degree to which chronic, systemic inflammation leads to low BMD and bone disease in patients with chronic HCV infection has not been investigated. Therefore, in this study, we aimed to evaluate the association between systemic inflammatory markers, BMD, and markers of bone turnover in a well-characterized cohort of patients with chronic HCV infection without cirrhosis.

Methods

Subjects

This was a cross-sectional study of patients with chronic HCV infection, defined by two detectable HCV RNA levels at least 6 months apart. Only patients between the ages of 40–60 years were included, as this age range represents a group at higher risk of bone loss (compared with <40 years), but would not typically be considered for osteoporosis screening in clinical practice according to guidelines from the US Preventive Services Task Force [25]. Stage of liver disease was confirmed by either:

1. Liver biopsy within 1 year of enrollment showing stages 1, 2, or 3 fibrosis on the Batts–Ludwig scoring system [26] or

2. The combination of the following three criteria:

   a. Platelet count >140,000 per μL

   b. Lack of clinical history of complications of end-stage liver disease (e.g., varices, ascites, or hepatic encephalopathy), and

   c. Lack of radiographic evidence of cirrhosis and portal hypertension as defined by (a) the absence of nodularity of the liver edge, (b) the absence of splenomegaly, (c) portal vein diameter>12 mm, or (d) the presence of intra-abdominal collaterals.

Patients were excluded if they were currently taking any anti-resorptive or anabolic medications for known bone disease, had any comorbidities that influence bone metabolism (e.g., prior organ transplantation, use of chronic immunosuppression, chronic kidney disease

with estimated glomerular filtration rate (eGFR) <60 mL/min by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [27], or current alcohol dependence defined as >4 drinks/day for men and >2 drinks/day for women), were currently undergoing treatment for chronic HCV, or had any contraindications to undergoing DXA scan.

Study Procedures, Data Collection, and Data Classification

All patients completed a questionnaire to obtain demographic information, general medical history, HCV-related history, current medications, menstrual history (for women only), lifestyle factors including alcohol and tobacco use, weekly exercise, and family history of osteoporosis or atraumatic fracture. Basic laboratory data that were drawn as part of routine clinical care within 6 months of the study visit were obtained from a retrospective review of the patients' electronic medical records. These laboratories included: platelet count, creatinine, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, calcium, phosphorus, and HCV viral load.

All subjects underwent one measurement of BMD at the lumbar spine, left femoral neck, and left total hip by DXA scan as part of the study protocol independent of any clinical indication. All DXA scans were performed on the same bone densitometer (Lunar enCORE™; GE Medical Systems, Madison, WI). Reference values for age-, sex-, and race-matched controls were obtained from the DXA manufacturer's database. Osteopenia was defined as a *T* score between −1.0 and −2.5 and osteoporosis was defined as a *T* score −2.5, according to the standard definition from the World Health Organization [28].

On the same day, all subjects had one blood sample measurement. These samples were immediately aliquoted and frozen. At the end of the study, all samples were processed in a single batch to obtain the following results using assays available from the UK Immunodiagnostic Systems, Ltd: total alkaline phosphatase, 25(OH) vitamin D, intact parathyroid hormone (iPTH), bone-specific alkaline phosphatase (BSAP, a marker of bone formation), intact procollagen type I amino-terminal propeptide (P1NP, a marker of bone formation), serum C-terminal cross-linking telopeptide of type I collagen (CTx, a marker of bone resorption), high-sensitivity C-reactive protein (hsCRP), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6).

Markers of systemic inflammation were categorized as elevated based on the upper limits of normal for the reference values for each assay: CRP 3 mg/L, TNF-α 6 pg/mL, and IL-6 pg/mL. A 25(OH) vitamin D level 20 ng/mL was classified as deficient [29]. ALT was categorized into three groups: 42 U/L (the upper limit of normal for this assay), between 43 and 74 U/L, and 75 U/L (75th percentile for the cohort).

Statistical Analysis

The primary outcome was the presence of low BMD, defined as a *T* score −1.0. Primary predictors were markers of systemic inflammation (e.g., hsCRP, TNF-α, and IL-6). Secondary predictors were markers of bone turnover (e.g., serum calcium × phosphorus product, iPTH, BSAP, P1NP, and serum CTx).

Chi-square tests, *t* tests, and one-way ANOVA were used to compare characteristics between groups. *Z* scores for BMD were calculated using the reference data for age-, sex-, and race-matched controls available from the DXA manufacturer's database. *Z* scores for BSAP, CTX, and P1NP were calculated using the reference data available for each specific assay. Logistic regression assessed the association between the predictors of interest and the presence of low BMD.

This study was approved by the University of California, San Francisco Committee on Human Research (Institutional Review Board). All procedures followed were in accordance with the ethical standards of the UCSF Committee on Human Research and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients to be included in this study.

Results

Baseline characteristics of the 60 subjects with chronic HCV without evidence of cirrhosis are listed in Table 1, column A. The mean age was 53.3 years, 53 % were female, 53 % were non-Hispanic White, and mean body mass index (BMI) was 28.4 kg/m^2. Prior tobacco use was reported in 53 %, but only 25 % were currently using tobacco, 35 % reported using alcohol heavily in the past, and 20 % had a family history of osteoporosis. Mean ALT was 60.3 U/L, with only 33 % having an ALT less than the upper limit of normal (42 U/L). Mean creatinine was 0.8 mg/dL with mean eGFR of 93.9 mL/min. Sixty-seven percent had fibrosis stage <2 with a mean HCV viral load of 6.0 log IU/mL; 23 % had previously undergone HCV treatment. At the time of enrollment, 17 % reported taking calcium supplements, 25 % vitamin D, 12 % proton-pump inhibitor, and 7 % estradiol.

A total of 25 (42 %) subjects had low BMD—18/25 (30 %) had osteopenia and 7/25 (12 %) had osteoporosis. Baseline characteristics of those with normal and low BMD are shown in columns B and C, respectively, in Table 1. Compared with those with normal BMD, HCV-infected subjects with low BMD had lower BMI (26.2 vs. 29.9, $p = 0.03$) and higher rates of calcium supplementation (28 vs. 9 %, $p = 0.05$), but were otherwise similar with respect to all other baseline characteristics. Mean (SD) BMD of the lumbar spine among the 60 subjects with chronic HCV was 1.098 (0.398) g/cm^2 with a mean *T* score of −0.5 (1.5). Mean (SD) *Z* score of the lumbar spine in the HCV-infected patients was −0.4 (1.4), which was significantly different compared with age-, sex-, and race-matched controls available in the DXA manufacturer's database [$p = 0.02$]. At the left femoral neck, mean (SD) BMD was 0.962 (0.149) g/cm^2 with a mean (SD) *T* score of −0.5 (1.2). Mean (SD) *Z* score at the left femoral neck was −0.2 (1.0) [$p = 0.14$ compared with age-, sex-, and race-matched controls in the DXA manufacturer's database]. At the total hip, mean (SD) BMD was 1.060 (0.161) g/cm^2 with a mean (SD) *T* score of −0.2 (1.0) and *Z* score of −0.2 (1.0) [$p = 0.22$].

Markers of Inflammation and Bone Turnover

Table 2 details the levels of inflammatory markers, and serum markers of bone metabolism and turnover. With respect to inflammatory markers, there were no significant differences in ALT, high-sensitivity CRP, TNF-α, or IL-6 among those with and without low BMD. Subjects with low BMD had statistically significantly higher levels of serum phosphorus

(4.1 vs. 3.5 mg/dL; $p = 0.04$) and P1NP (73.1 vs. 47.5 ng/mL; $p < 0.01$) compared with subjects with normal BMD. 25(OH) vitamin D levels were similar between the two groups (27.8 vs. 31.0 ng/mL; $p = 0.42$). Even after excluding patients taking vitamin D supplementation, 25(OH) vitamin D levels were still similar between groups (24.0 vs. 29.0 ng/mL; $p = 0.87$). Serum calcium (9.1 vs. 9.3 mg/dL; $p = 0.08$) was numerically lower and serum calcium–phosphorus product (37.1 vs. 33.3; $p = 0.08$), bone-specific alkaline phosphatase (24.7 vs. 21.6 U/L; $p = 0.18$), and CTx (443.9 vs. 331.1 pg/mL; $p = 0.12$) were numerically elevated among those with low BMD, but these differences did not meet statistical significance.

Associations Between Markers of Inflammation, Markers of Bone Turnover, and Low BMD

We then evaluated associations between elevated markers of inflammation or bone turnover with the presence of low BMD using logistic regression. In univariable analysis, serum phosphorus levels (OR 3.33; 95 % CI 1.05–10.53; $p = 0.04$) and P1NP (OR 1.04 per unit; 95 % CI 1.01–1.08; $p < 0.01$) were associated with a statistically significantly increased odds of low BMD. There was a trend ($p <0.2$) toward an association with low BMD for serum calcium level (OR 0.27 per unit; 95 % CI 0.06–1.26; $p = 0.10$), calcium–phosphorus product (OR 1.10 per unit; 95 % CI 0.98–1.23; $p = 0.12$), and bone-specific alkaline phosphatase (OR 1.05 per unit; 95 % CI 0.98–1.11; $p = 0.18$). There were no significant associations between elevated ALT levels 75 U/L (OR 0.90; 95 % CI 0.23–3.47; $p = 0.88$), hsCRP (OR 1.04 per unit; 95 % CI 0.74–1.47; $p = 0.82$), TNF-α (OR 1.02 per unit; 95 % CI 0.96–1.08; $p = 0.56$), or IL-6 (OR 1.00 per unit; 95 % CI 0.99–1.01; $p = 0.56$) with low BMD. In multivariable logistic regression, after adjustment for female gender and BMI (given the clinical importance of both characteristics to BMD), only P1NP remained statistically significant predictor of low BMD (OR 1.04 per unit; 95 % CI 1.01–1.08; $p = 0.01$).

Discussion

In light of the recent recommendation by the Centers for Disease Control and US Preventive Services Task Force that all individuals born between 1945 and 1965 be screened for chronic HCV [30, 31], the population of patients diagnosed with chronic HCV is expected to rise sharply [1, 32]. While the current era of direct-acting anti-HCV agents offers the hope of cure for many of these individuals, understanding the long-term sequelae of chronic HCV infection, such as hepatic osteodystrophy, even after sustained virologic response is ever more important.

In this study, we report that low BMD is highly prevalent among individuals between the ages of 40 and 60 years with chronic HCV infection without cirrhosis. As measured by DXA scan, 42 % had low BMD by DXA scan, and 12 % met criteria for osteoporosis. These rates are substantially higher than rates reported in the general population in this<60 year age group [33, 34]. In addition, we found that two bone and mineral parameters—serum phosphorus and P1NP—may be useful biomarkers for identifying the subgroup of patients with chronic HCV at risk for accelerated bone loss.

The rate of osteoporosis (~12 %) that we observed in our cohort is similar to those seen among patients with chronic inflammatory conditions, such as ulcerative colitis [35],

ankylosing spondylitis [36], and rheumatoid arthritis [37, 38]. Despite the common association between chronic inflammatory illnesses and low BMD, we did not find a statistically significant relationship between inflammatory markers and BMD in our HCV-infected subjects. The average levels of systemic inflammation as measured by high-sensitivity CRP, TNF-α, or IL-6 were normal in our cohort and lower than expected based on prior studies evaluating systemic inflammatory markers in individuals with chronic HCV [14, 15]. Our data suggest that a mechanism other than ongoing chronic systemic inflammation explains the high prevalence of low BMD in this population.

We acknowledge several limitations to this study. First, serum measurements were drawn at a single time point, potentially missing associations between inflammatory markers, bone turnover markers, and BMD that occurred earlier in this chronic disease. It is theoretically possible that the initial inflammatory response to acute HCV infection contributes to early bone loss, but was not detected in this cross-sectional study. Second, we were unable to obtain accurate information on disease duration for the majority of patients, as most patients reported that their HCV risk factor was ''unknown.'' However, the major risk factors for HCV are injection drug use (usually in an individual's young adulthood) and blood transfusion before 1992, so we assume—as has been demonstrated in other studies [1]—that the duration of infection was at least 20 years for most patients. We did not obtain information regarding duration of use of potential confounding bone-active medications including estrogen and proton-pump inhibitors, but use of these medications occurred in relatively low proportions in this cohort and did not differ significantly between those who had low BMD and those who did not. Lastly, phlebotomy was not consistently performed in the fasting state or in the morning, which may have resulted in slight inter-individual variations in measurements of bone turnover markers and may have influenced the serum phosphorus levels [39].

In conclusion, chronic HCV infection is associated with low BMD even in the absence of cirrhosis. Our data suggest that BMD assessments should occur at a younger age in both men and women with chronic HCV infection with the goal of implementing interventions targeted at reducing BMD loss or even improving BMD before the onset of debilitating and potentially life-threatening fractures. Serum markers of bone turnover, such as P1NP, may be useful to identify the subgroup of chronic HCV-infected individuals who are at highest risk of accelerated bone loss and should be the focus of further study.

Acknowledgments

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Table 1

Baseline characteristics of the 60 non-cirrhotic patients with chronic HCV

Characteristic[a]	A	B	C	D
	All	Normal BMD	Low BMD	p value
	n = 60	n = 35 (58 %)	n = 25 (42 %)	
Age (years)	53.3 (5.7)	52.6 (5.6)	54.2 (5.8)	0.27
Female	32 (53 %)	16 (46 %)	16 (64 %)	0.16
Postmenopause[b]	24 (75 %)	8 (50 %)	16 (67 %)	<0.01
Race/ethnicity				0.32
Non-Hispanic White	32 (53 %)	15 (43 %)	17 (68 %)	
African-American	15 (25 %)	11 (31 %)	4 (16 %)	
Hispanic White	11 (18 %)	7 (20 %)	4 (16 %)	
Other	2 (3 %)	2 (6 %)	0 (0 %)	
Type II diabetes	17 (28 %)	9 (26 %)	8 (32 %)	0.59
Tobacco use ever	32 (53 %)	21 (60 %)	11 (44 %)	0.22
Current tobacco use	15 (25 %)	7 (20 %)	8 (32 %)	0.29
Heavy alcohol use ever	21 (35 %)	14 (40 %)	7 (28 %)	0.34
Family history of osteoporosis	12 (20 %)	6 (17 %)	6 (24 %)	0.51
Body mass index	28.4 (6.5)	29.9 (7.0)	26.2 (5.1)	0.03
Total bilirubin (mg/dL)	0.7 (0.3)	0.7 (0.4)	0.7 (0.3)	0.52
Creatinine (mg/dL)	0.8 (0.2)	0.9 (0.1)	0.8 (0.2)	0.08
eGFR (mL/min)	93.9 (14.4)	93.3 (13.9)	94.8 (13.9)	0.69
Albumin (g/dL)	4 (0.3)	3.9 (0.4)	4.0 (0.3)	0.45
Platelet count (10^9/L)	206 (54)	204 (62)	208 (62)	0.77
Had a liver biopsy within 1 year of enrollment	32 (53 %)	17 (49 %)	15 (60 %)	0.38
Fibrosis stage 2	14 (33 %)	8 (35 %)	6 (30 %)	0.74
Log HCV RNA (IU/mL)	6.0 (0.6)	6.1 (0.7)	6.0 (0.4)	0.39
Prior HCV treatment	14 (23 %)	8 (23 %)	6 (24 %)	0.92
Medication use				
Calcium	10 (17 %)	3 (9 %)	7 (28 %)	0.05
Vitamin D	15 (25 %)	10 (29 %)	5 (20 %)	0.45
Proton-pump inhibitor	7 (12 %)	5 (14 %)	2 (8 %)	0.46
Estrogen (women only)	2 (7 %)	1 (3 %)	1 (4 %)	0.81

[a] Mean (standard deviation) or n (%)

[b] n (% of the 32 women)

Table 2

Comparison of serum markers of inflammation and bone turnover between those normal BMD and those with hepatic osteodystrophy (e.g., osteopenia/osteoporosis)

Marker[a]	All n = 60	Normal BMD n = 35	Low BMD n = 25	p value
Inflammation				
ALT (U/L)	60.3 (35.0)	62.6 (37.0)	57.0 (33.7)	0.55
% with ALT 42 U/L	20 (33 %)	12 (34 %)	8 (32 %)	0.86
% with ALT 43–74 U/L	24 (40 %)	13 (37 %)	11 (44 %)	
% with ALT 75 U/L	16 (27 %)	10 (29 %)	6 (24 %)	
High-sensitivity CRP (mg/L)	0.8 (1.5)	0.7 (1.0)	0.8 (2.0)	0.82
% with elevated hsCRP (3 mg/L)	3 (5 %)	2 (6 %)	1 (4 %)	0.76
TNF-α (pg/mL)	5.2 (9.0)	4.6 (8.1)	5.9 (10.2)	0.57
% with elevated TNF-α (6 pg/mL)	16 (26 %)	8 (22 %)	8 (31 %)	0.43
IL-6 (pg/mL)	21.0 (68.3)	16.5 (40.4)	27.4 (95.3)	0.55
% with elevated IL-6 (7 pg/mL)	20 (32 %)	12 (33 %)	8 (31 %)	0.96
Bone turnover, calciotropic hormones and minerals				
Calcium (mg/dL)	9.3 (0.5)	9.3 (0.5)	9.1 (0.4)	0.08
Phosphorus (mg/dL)	3.8 (0.9)	3.5 (0.5)	4.1 (1.2)	0.04
Calcium–phosphorus product	34.8 (8.9)	33.0 (4.5)	37.1 (12.0)	0.08
25(OH) vitamin D (ng/mL)	29.6 (14.9)	31.0 (16.3)	27.8 (12.9)	0.42
% with 25(OH)D <20 ng/mL	18 (30 %)	9 (26 %)	9 (36 %)	0.39
Total alkaline phosphorus (U/L)	74.3 (22.0)	72.2 (18.1)	77.3 (26.6)	0.38
iPTH (pg/mL)	42.3 (62.5)	46.7 (81.1)	36.1 (14.9)	0.52
Bone-specific alkaline phosphorus (U/L)	22.9 (8.6)	21.6 (7.4)	24.7 (9.9)	0.18
Z score BSAP	1.8 (1.5)	1.8 (1.6)	2.0 (1.5)	0.68
C-telopeptide (pg/mL)	378.1 (277.7)	331.1 (253.7)	443.9 (301.1)	0.12
Z score C-telopeptide	−0.8 (1.2)	−0.9 (1.3)	−0.7 (1.0)	0.59
P1NP (ng/mL)	58.2 (35.2)	47.5 (15.7)	73.1 (48.0)	<0.01
Z score P1NP	−0.8 (1.4)	−1.2 (0.6)	−0.2 (1.9)	<0.01

[a]Mean (standard deviation) or *n* (%)