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Bone alterations in hepatitis C virus infected patients

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ABSTRACT

Background and aims: Most studies have shown that patients with chronic hepatitis C virus (HCV) infection are affected by osteoporosis. However, liver function impairment and deranged nutrition may both play a role in the bone alterations observed. In some works no osteoporosis was found, and some cases of osteosclerosis have been reported. The aim of the study is to assess bone alterations in treatment-naïve, well-nourished HCV patients, in order to discern whether or not HCV infection causes osteoporosis.
Methods: Whole-body bone densitometry and assessment of T-score at lumbar spine and hip were performed to 40 patients and 40 age- and sex-matched controls, with a Lunar Prodigy Advance (General Electric, Piscataway, NJ, USA). All the patients underwent liver biopsy. Nutritional evaluation was performed by subjective nutritional assessment, body mass index (BMI), and densitometric assessment of total lean mass and total fat mass. Serum osteocalcin, osteoprotegerin, RANKL, PTH, crosslaps, vitamin D3, testosterone, IGF-1, and estradiol were determined.
Results: Patients did not show differences in total bone mineral density (BMD) or T-score with controls. On the contrary, about a third of them showed positive T scores. Patients showed lower IGF-1, vitamin D3 and testosterone, but higher telopeptide levels, and a trend to higher osteoprotegerin levels. Multivariate analyses disclosed that age, sex, and total lean mass were the only parameters independently related with BMD.
Conclusions: Therefore, chronic HCV infection in well nourished patients with preserved liver function does not cause osteoporosis.

1. Introduction

Hepatitis C virus (HCV) infection is a chronic viral disease caused by the flavivirus hepatitis C virus. Although sometimes associated to multisystemic manifestations, liver disease is the most common affectation, and leads to liver cirrhosis and hepatocarcinoma. It is very frequently found among alcoholics [1], so that both injury factors – ethanol and HCV infection– usually co-exist in the same patient, being difficult to discern which of both is responsible for the clinical and pathological features presented by these patients. In HCV infection, osteopenia has been described, but many factors, some of which are commented below, may a play a role.

Chronic alcohol consumption is associated with osteopenia, mainly due to both direct and indirect effects of ethanol on bone remodeling in a dose-dependent fashion [2–7]. The frequently associated malnutrition in these patients also contributes [8]. Superimposed to these factors, the peculiar style of life of the alcoholic, prone to falls, traffic accidents, and violence, leads to an increase in bone fractures [9].

Osteopenia has been also described in other forms of liver disease, especially cholestatic ones [10]. Bilirubin may impair osteoblastic function [11], but liver failure is even more important in its pathogenesis [12,13].

Osteopenia is also common in chronic HCV patients. In most studies, data suggest that HCV by itself provokes osteopenia [13–17]. Some of this research involved non-cirrhotic patients [17–20], others, individuals affected by liver cirrhosis [13,14,16], or both cirrhotics and non-cirrhotics [21,22], and some were restricted to patients awaiting organ transplantation [16,23], in whom liver function derangement and/or altered nutritional status surely played a role. Therefore, in contrast with ethanol, for which clinical and experimental data clearly show a direct effect on osteopenia in alcoholics, the exact role of HCV infection in the altered bone mineral density (BMD) observed in chronic HCV infected patients is not fully known. Moreover, osteosclerosis has been described in some case reports on patients with chronic HCV infection [24–27]. In addition, some relatively large series fail to detect significant bone alterations in HCV patients [19], at least attributable to HCV [28].

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Based on these facts, we performed this study in order to assess bone mineral density and biochemical variables involved in bone homeostasis in chronic HCV-infected, non-alcoholic, well-nourished patients with a still preserved liver function, and without a co-infection with the human immunodeficiency virus (HIV), comparing the results with those obtained in an age-, and sex-matched control group composed by non-alcoholic, non-HIV infected, well nourished individuals.

2. Methods

2.1. Patients and controls

We included 40 patients (14 women, 4 menopausic) affected by HCV, aged 41.80 ± 10.40 years, and 40 age- (42.45 ± 9.50 years, t = 0.29) and sex-matched (7 women, $\chi^2 = 3.12$; p = 0.08; 2 menopausic) controls, hospital workers, drinkers of less than 10 g/day. Diagnostic criteria for HCV infection were the following two: (1) presence of anti-HCV and/or HCV RNA by reverse transcriptase polymerase chain reaction (PCR), and (2) histology consistent with HCV. Most patients (32) were infected by HCV type 1 genotype, 4 by type 3 genotype, 3 by type 4, and one case showed infection by several genotypes. All these patients were consecutively recruited before a treatment for hepatitis C virus was administered. Since in many cases, HCV infection remains asymptomatic for a long time, we cannot know during how long the infection was present. However, twenty patients were already aware that they were HCV positive, since a median time of 10 years (interquartile range = 2–14.5 years).

Serological tests for detection of human immunodeficiency virus (HIV) infection were performed to all the patients. A negative HIV test was required for inclusion in this study.

2.2. Bone densitometry

After informed consent, the 40 patients and 40 controls underwent densitometric evaluation with a Lunar Prodigy Advance device (General Electric, Piscataway, NJ, USA). Two kinds of explorations were performed: a specific bone densitometric study of hip and lumbar spine, in order to record Z and T scores, following standard criteria [29], and a whole body densitometric analysis, recording bone mineral density (BMD), fat, and lean mass at different parts of the body, such as the upper limbs, ribs, pelvis, lower limbs, spine, and total body. Total lean mass and total fat mass were used in this study in the assessment of nutritional status.

2.3. Nutritional and dietary evaluation

Following a previously reported protocol [8], we recorded the eating habits of the patients, asking them where do they usually eat (if at home or in bars or taverns), how many times a day, and what they eat (sandwiches or snacks, or normal dishes) classifying them in three categories: normal, irregular habits (loss of some meals), and poor eating habits (usually in bars or taverns, in form of sandwiches or snacks, once or twice daily). Since only 2 patients fell in this third category, we further grouped our patients in only 2 groups (normal vs irregular eating habits).

In addition to body mass index (BMI, as weight (in kg)/height^2 (in m)), and to total lean and total fat mass densitometric assessment, subjective nutritional evaluation was performed to all the patients, as follows: we examined the muscle masses of the upper and lower limbs and of the temporal muscle, defining two degrees of atrophy (severe, moderate) and absence of atrophy, and assigned 2, 1 and 0 points to each category, respectively. We also recorded, by physical examination, the fat loss on the cheek and abdomen, Bichat's fat and subcutaneous fat atrophy, and classified them in a similar way, defining a score (SNS), based on the sum of the assigned points, for which the poorest value was 10 and 0 the best one. We further classified our patients in well-nourished (0–2 points), moderately undernourished (3–4 points) and severely undernourished (5–10 points), since this classification is related to prognosis [30].

2.4. Biochemical assessment

In addition to routine laboratory evaluation (which included, among other variables, bilirubin, prothrombin activity, and serum albumin, Table 1), we performed the following biochemical determinations: serum osteocalcin, to all the patients and controls, by immunometric chemiluminescent assay (recovery = 97–121%; variation coefficients of assays ranging from 3.5% to 7.1%; DPC, Los Angeles, CA, USA), as a marker of bone synthesis, and C-terminal telopeptide of type I collagen (CrossLaps), by one step ELISA, with a recovery ranging from 94 to 107% and an intra- and interassay variation coefficient ranging 4.7–4.9% and 5.4–8.1%, respectively (Osteometer BioTech A/S, Herlev, Denmark), as a marker of bone breakdown. This parameter was determined only to 28 patients and the 40 controls. We also determined serum IGF-1 (chemiluminescent assay, DPC, Los Angeles, CA, USA), to 39 patients and all the controls; 1,25 dihydroxyvitamin D3 to 27 patients and all the 40 controls (radioimmunoassay (RIA), Nichols, San Juan Capistrano, CA, USA); parathyroid hormone (PTH), to 36 patients and 19 controls; serum testosterone to 26 patients and 33 controls (only men, solid phase RIA); serum RANKL, to 26 patients and 18 controls, by ELISA, with a sensitivity of 0.08 pmol/l and a variation coefficient of 5% or less (intra-assay) and 9% or less (Immundiagnostik, Bensheim, Germany); and osteoprotegerin (OPG), to 34 patients and 24 controls, by sandwich enzyme-linked immunosorbent assay (ELISA), with a sensitivity of 0.14 U/l, and intra-assay and inter-assay variation coefficients <10% (BioVendor, Brno, Czech Republic), and also estradiol (solid phase RIA), to 38 patients and 40 controls.

All these data were recorded the day at which the patients underwent a liver biopsy before receiving active treatment against HCV infection.

The study protocol was approved by the local ethical committee of our Hospital and conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

2.5. Statistics

The Kolmogorov–Smirnov test was used to test for normal distribution. Student's t test was used to compare mean values among two different groups (i.e., men and women, controls and patients), whereas Spearman's ρ and Pearson's correlation were utilized to compare quantitative variables among each other. Also, χ^2 square test was used to compare qualitative variables with each other.

Table 1
Some clinical variables in the three groups analyzed. Results are given as mean ± standard deviation; ANOVA test (F) was used for comparisons among groups.

	HCV non-drinkers (40)	Controls (40)	T; p
Age (years)	41.80 ± 10.40	42.45 ± 9.50	T = 0.29; NS
Body mass index (BMI) (kg/m^2)	24.42 ± 3.14	25.64 ± 3.66	T = 1.60;NS
Albumin (g dl^{-1})	4.34 ± 0.39	4.27 ± 0.17	T = 0.69; NS
Bilirubin (mg dl^{-1})	0.81 ± 0.64	0.88 ± 0.10	T = 0.73; NS
Prothrombin activity (%)	95.82 ± 9.22	98.90 ± 2.33	T = 0.82;NS
Spine BMD (g cm^{-2})	0.88 ± 0.13	0.88 ± 0.10	T = 0.90; NS
Legs BMD (g cm^{-2})	1.27 ± 0.14	1.35 ± 0.11	T = 2.77; p = 0.007
Ribs BMD (g cm^{-2})	0.66 ± 0.06	0.71 ± 0.06	T = 3.43; p = 0.001
Arms BMD (g cm^{-2})	0.88 ± 0.11	0.84 ± 0.07	T = 1.79; NS
Total BMD (g cm^{-2})	1.15 ± 0.10	1.17 ± 0.10	T = 0.67; NS
Total hip T-score	−0.40 ± 1.16	−0.04 ± 1.12	T = 0.70; NS
Lumbar spine T-score	−0.78 ± 1.24	−0.34 ± 1.29	T = 0.49; NS
Total fat mass (g)	20,612 ± 11,484	20,680 ± 8413	T = 0.03; NS
Total lean mass (g)	47,069 ± 9194	53,098 ± 6328	T = 3.37; p<0.001