https://doi.org/10.31925/farmacia.2022.2.13 **ORIGINAL ARTICLE**

REDUCED BONE MINERAL DENSITY IN YOUNG, NON-CIRRHOTIC PATIENTS WITH CHRONIC VIRAL HEPATITIS

GEORGEANA ȚUCULEANU [1#], ECATERINA CONSTANȚA BARBU [1,2*], MIHAI LAZĂR [1,2#], CRISTINA EMILIA CHIȚU-TIȘU [1,2], CRISTINA MIHAELA OLARIU [1,2], MIHAI BOJINCĂ [1,3], CĂTĂLIN TILIȘCAN [1,2], ȘTEFAN SORIN ARAMĂ [1,2], VICTORIA ARAMĂ [1,2#], DANIELA ADRIANA ION [1,2]

[1] *"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*
[2] *"Prof. Dr. Matei Balș" National Institute for Infectious Diseases, Bucharest, Romania*
[3] *"Dr. I. Cantacuzino" Clinical Hospital, Bucharest, Romania*

[#] *Authors with equal contribution*
**corresponding author: katybarbu@yahoo.com*

Manuscript received: October 2021

Abstract

Bone anomalies are well-known complications of advanced hepatic diseases, but a relationship with non-cirrhotic hepatopathies remains to be confirmed. In this study, we investigated young adults with viral hepatitis B or C to determine whether their condition may impact bone loss and which factors influence this phenomenon. Subjects were divided in 3 groups. Descriptive statistics were based on anthropometric, bone density and disease-related parameters (viral load, disease length, fibrosis grade and treatment history). We compared bone demineralization between each group and aimed to determine associations between anthropometric or disease-related parameters and bone density measurements. Multiple linear regression analysis was performed to identify predictors for bone demineralisation. Reduced bone mineral density prevalence was of 11%, most of them males in hepatitis B group and, respectively 11%, most of them females in hepatitis C group, 5 times greater than the control group. Hepatitis C females had lower Z-scores than control females at the hip. In hepatitis B patients, age, male gender, fibrosis grade, disease length and antiviral therapies were found to play an important role in bone demineralization. In hepatitis C patients, most of the anthropometric characteristics (body mass index and female gender) had quantifiable impact on bone loss. These patients could be considered for early screening and treatment.

Rezumat

Demineralizarea osoasă este o complicație cunoscută a afecțiunilor hepatice avansate, dar prezența acesteia în stadiile pre-cirotice nu a fost confirmată încă. În acest studiu, am investigat pacienți tineri cu hepatită virală B sau C pentru a determina dacă patologia lor influențează mineralizarea osoasă și ce factori contribuie la acest proces. Pacienții selectați au fost incluși în 3 grupuri de studiu. S-au realizat statistici descriptive bazate pe parametrii antropometrici, osteodensitometrici și legați de boală (încărcătura virală, durata bolii, gradul fibrozei și istoricul de tratament). S-a comparat mineralizarea osoasă între cele 3 grupuri și, prin analiza corelațiilor, am investigat asocierile dintre parametrii antropometrici și legați de boală și mineralizarea osoasă. O analiză de regresie liniară multiplă a determinat factorii de predicție ai demineralizării. Prevalența demineralizării a fost de 11%, majoritatea bărbați, în grupul cu hepatită B și de 11%, majoritatea femei, în grupul cu hepatită C, de 5 ori mai mare față de grupul control. Femeile cu hepatită C au avut scoruri Z mai mici decât femeile control la nivelul șoldului. În cazul hepatitei B, vârsta, sexul masculin, gradul fibrozei, durata bolii și tratamentul antiviral au avut un impact negativ asupra mineralizării. În cazul hepatitei C, parametrii antropometrici (indicele de masă corporală și sexul feminin) influențează demineralizarea. Acești pacienți trebuie avuți în vedere pentru screening și tratament precoce.

Keywords: bone mineral density, viral hepatitis, non-cirrhotic patients

Introduction

The estimated global prevalence of chronic viral hepatitis in 2018 was of 257 million for hepatitis B virus (HBV) and 71 million for hepatitis C virus (HCV), thus reinforcing their position as a major health concern both among physicians and researchers [19, 20]. Considering the substantial progress that has been made in effectively treating viral hepatitis, we are now facing the challenges of long-term management of these patients and the complications caused by the extensive course of their liver disease. Osteoporosis has been stated as one of the consequences of chronic liver diseases, especially in advanced cirrhosis (also known as *Hepatic Osteodystrophy)* and cholestatic disease, but evidence in favour of more prevalent illnesses such as viral hepatitis, NASH (non-alcoholic steatohepatitis) or alcoholic liver disease causing low bone mineral density (BMD) have only emerged in the last 2 - 3 decades [1, 16, 17].

The mechanisms behind this association remain only partly described, but lately, several hypotheses have

been proposed. Dysfunction of the growth hormone (GH) – insulin-like growth factor 1 (IGF-1) signalling pathway seems to be a molecular consequence of both HCV and HBV chronic infection and has been associated by some authors with low BMD [1, 22, 23]. The chronic inflammation that accompanies viral hepatitis has also been incriminated for the loss of bone mass; specifically, interleukin 6 (IL-6) and tumour necrosis factor α (TNF-α) - cytokines known for their role in hepatic lesions of viral aetiology - were linked to an increase in osteoclast activity *via* receptor activator of nuclear factor kappa-β ligand (RANKL) stimulation [12, 18].

However, patients with chronic liver diseases - including viral hepatitis - exhibit several independent risk factors for osteoporosis such as: alcohol abuse, malnutrition and immobility that may contribute to the outcome on BMD. Moreover, in this context, diagnosing osteoporosis can be challenging since early on, very few clinical features are present (vertebral fractures are the most frequent complications, but often remain asymptomatic), while more severe consequences, such as femoral neck fractures only appear after a longer evolution (more than 10 years) [6]. This delay usually exceeds the life expectancy of cirrhotic patients, but not that of viral hepatitis patients.

Following this hypothesis, several authors have investigated whether chronic infection with HBV/HCV could result in bone loss and which additional factors may promote or protect from this complication. Summarizing the results, the prevalence of osteoporosis in this population varies between 3% and 36% depending on the site of dual X-ray absorptiometry (DXA) measurements, the age of the participants, the inclusion of either postmenopausal women or cirrhotic patients [2, 26, 27].

In this study, we aimed to supplement the data in the literature related to the impairment of bone mineralization in a specific subtype of subjects – non-cirrhotic, young (men under 50 years and premenopausal women) individuals with chronic HBV or HCV infection and without comorbidities. Thus, the main objective of this study is to determine if there is an association between HBV and/or HCV chronic infections and low BMD in the absence of other known risk factors and to describe the phenomenon and the variables involved considering a population of young adults where important preventive measures could successfully be established.

Materials and Methods

Study population and design

We performed a cross-sectional study between 2015 and 2016. 132 patients with chronic viral hepatitis B (n = 66) or C (n = 66) were compared to 65 healthy controls.

The inclusion criteria were: (1) persistently positive (≥ 6 months) HBsAg and/or (2) histological diagnosis for HBV chronic infection and (1) the presence of anti-HCV antibodies and/or HCV ribonucleic acid (RNA) by polymerase chain reaction (PCR) for over 6 months or (2) histology consistent with HCV infection for HCV chronic infection.

The exclusion criteria were menopause (physiological/ surgical), human immunodeficiency virus (HIV) co-infection, autoimmune hepatitis, bone diseases, vitamin D deficiency, endocrine diseases, the use of drugs that interfere with bone metabolism (corticosteroids for over 3 months, osteoporosis medication), advanced illnesses such as: severe heart disease, chronic kidney disease (estimated glomerular filtration rate (eGFR) < 50 mL/min/1.73 m^2), neoplasia; malnutrition, prolonged immobilization. None of the patients or healthy subjects had a family history of osteoporosis and all of them were engaged in moderate physical activity (at least 30 minutes of activity equivalent to brisk walking or jogging more than 3 times/week).

Body weight and height were measured for each participant and used to calculate the BMI (body mass index) (kg/m^2). Subjects were then classified based on their nutritional status (underweight, normal weight, overweight, obese) according to WHO (World Health Organization) criteria [31].

Ethical statements

Ethical approval was obtained from the Ethics Committee of the institute according to the ethical and moral principles stated in the Helsinki 1975 Declaration on Human Rights. All patients were over 18 years old and signed the informed consent forms prior to their enrolment in the study.

Bone density evaluation

We performed BMD in all subjects at the lumbar spine (L1 - L4), femoral neck and total hip using a DXA machine (GE LUNAR DPX-NT, General Electric Company, New York, Connecticut, USA) as indicated by the manufacturer. For femoral neck and total hip measurements, we included in the analysis the data from the more affected side.

Considering our study population of men under 50 years of age and premenopausal women, we expressed the results according to the guidelines using the Z-score and BMD (g/m^2). Z-scores > -2 were considered as within the expected range for age, while Z-scores ≤ -2 were considered below the expected range for age [28].

Evaluation of the liver disease

We assessed several parameters regarding the severity and evolution of the chronic liver disease. Liver fibrosis was non-invasively evaluated using FibroTest (Bio-Predictive, Paris, France) and expressed as fibrosis scores ranging from 0 to 4. Viral loads for each patient were obtained and were classified as undetectable (< 2000 IU/mL for HBV and 0 IU/mL for HCV) or detectable (> 2000 IU/mL for HBV and > 0 IU/mL for HCV). History of antiviral therapy and disease

length since diagnosis were also gathered for each patient during the interview.

Treatment

According to local protocols and to treatment indication at the time of the enrolment, the patients with chronic HBV infection followed different treatment regimens; and some of them experienced even multiple regimens. Thus, they were exposed to pegylated interferon α 2a, standard interferon α, lamivudine, entecavir or tenofovir. Patients with chronic HCV infection also followed different treatment regimens: pegylated interferon α 2a, pegylated interferon α 2b, standard interferon α and ribavirin.

Statistical analysis

Descriptive statistics were used to analyse demographic, anthropometric and clinical data. For some analyses, subjects were divided in age categories: 20 - 29 years old, 30 - 39 years old and over 40 years old. Results are expressed as mean ± standard deviation for continuous variables and as percentages for categorical variables. The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to assess whether the data were normally distributed. Student t-tests or ANOVA were used to compare means, the Mann–Whitney U test for medians and the asymptotic Pearson's χ^2 test for percentages.

In order to compare the demineralization levels between groups (HBV infected patients *versus* HCV infected patients *versus* control group) and to investigate whether sex moderated this relationship, a One-way MANOVA with Post-hoc Bonferroni corrections was conducted. The Z-scores and BMDs (g/m^2) for lumbar spine, femoral neck and total hip were considered dependent variables.

Spearman's coefficient of correlation was used to estimate the association between BMD measurements and the following variables: age, nutritional status, fibrosis grade, viral load, history of treatment, disease length. The analyses were performed on the study groups as a whole and then split by sex.

In order to determine whether demineralization levels could be predicted for HBV and HCV infected patients while taking into consideration their sex, a series of multiple linear regressions were performed. Variables with a p-value < 0.20 in the bivariate analysis were included in the regression analysis. The adjusted R^2 (adjusted coefficient of determination) and the ANOVA were then used to assess the adequacy of the models.

Data were analysed using SPSS (SPSS Inc., Chicago, IL, USA) statistical software package version 27.0.1.0 for MacOS. The significance level was set at $p \leq 0.05$.

Results and Discussion

Characteristics of the study population

Relevant demographic and disease-related characteristics of the subjects enrolled are listed in Table I and Table II. The mean age of the patients was 37.27 ± 7.86 and 36.54 ± 7.42 for chronic hepatitis B and C groups respectively. 38/66 (58%) of HBV patients and 36/66 (55%) of HCV patients were males. The mean BMI was near the upper limit for normal weight i.e., 24.23 ± 3.49 for the HBV group and 24.15 ± 4.65 for the HCV group.

Most of the HBV patients (53/66 patients – 80%) had detectable viral load, while only 27/66 patients (41%) were positive for HCV RNA in the HCV group. The severity of the liver disease in the 2 groups was described in Table II using frequencies of fibrosis scores. The fibrosis grade of the patients was found to depend on their underlying hepatic infection. This relationship was also significant (p = 0.001) after we divided the groups in low-grade fibrosis (F0-F2) and high-grade fibrosis (F3-F4).

Table I

Demographic and anthropometric data of chronic hepatitis B and C patients and control group

	HBV (n = 66)	**Control (n = 65)**	**p**	**HCV (n = 66)**	**Control (n = 65)**	**p**
Age (years), (mean (SD))	37.27 (7.86)	36.54 (7.42)	0.58	36.98 (7.61)	36.54 (7.42)	0.74
Age group						
20 - 29, n (%)	14 (21%)	11 (16%)	0.76	12 (18%)	11 (16%)	0.98
30 - 39, n (%)	28 (42%)	27 (42%)		27 (41%)	27 (42%)	
> 40, n (%)	24 (36%)	27 (42%)		27 (41%)	27 (42%)	
Sex						
Females, n (%)	28 (42%)	31 (48%)	0.54	30 (45%)	31 (48%)	0.79
Males, n (%)	38 (58%)	34 (52%)		36 (55%)	34 (52%)	
BMI, kg/m^2, mean (SD)	24.23 (3.49)	23.84 (2.30)	0.45	24.15 (4.65)	23.84 (2.30)	0.62

Table II

Clinical and laboratory characteristics of chronic hepatitis B and C patients

	HBV (n = 66)	**HCV (n = 66)**	**p**
Viral load			
detectable, n (%)	53 (80%)	27 (41%)	**< 0.001**
undetectable, n (%)	13 (20%)	39 (59%)	

	HBV (n = 66)	HCV (n = 66)	p
Fibrosis grade			
F0, n (%)	14 (21%)	7 (11%)	**< 0.001**
F1, n (%)	25 (38%)	10 (15%)	
F2, n (%)	20 (30%)	27 (42%)	
F3, n (%)	6 (9%)	10 (15%)	
F4, n (%)	1 (2%)	12 (18%)	
Treatment status			
never treated	38 (58%)	21 (32%)	**0.003**
previously treated	28 (42%)	45 (68%)	
Disease length (years), median (range)	5 (2 - 9)	5 (2 - 10)	0.82

Data are expressed as means and standard deviations for continuous, normally distributed variables, frequencies for categorical variables and medians and interquartile ranges, 25^{th} - 75^{th} percentile for continuous variables with non-normal distribution. Significant p-values are highlighted

BMD comparison of the study groups and control group

BMD values (g/cm^2) and Z-scores of HBV, HCV and control subjects at all three sites considered in this study are listed in Table III as means ± SD. Low BMD for age (Z-score ≤ -2) was found in 7/66 (11%) patients with chronic HBV infection. Six patients with HBV infection - 6/66 (9%) - were diagnosed based on their lumbar spine mineral density and the other one based on total hip measurements. In the HCV group, 7/66 (11%) patients had low Z-scores for their age detected in the following skeletal sites: 3/66 (5%) lumbar spine, 4/66 (6%) total hip. Most of them were males in the hepatitis B group - 6 (9%) and females in the hepatitis C group - 5 (8%). In the control group only one male subject had a BMD ≤ -2 at lumbar spine

Table III

Bone mass measurements in hepatitis B and C patients and healthy subjects

	All patients			Males			Females		
	HBV (n = 66)	**HCV (n = 66)**	**Control (n = 65)**	**HBV (n = 38)**	**HCV (n = 36)**	**Control (n = 34)**	**HBV (n = 28)**	**HCV (n = 30)**	**Control (n = 31)**
BMD (g/cm^2), mean (SD)									
LS	1.14 (0.13)	1.15 (0.14)	1.13 (0.12)	1.14 (0.14)	1.17 (0.16)	1.17 (0.13)	1.15 (0.12)	1.13 (0.12)	1.1 (0.1)
FN	1.02 (0.14)	0.99 (0.13)	1.03 (0.1)	1.05 (0.14)	1.04 (0.13)	1.06 (0.08)	0.98 (0.14)	0.93 (0.1)	0.99 (0.1)
TH	1.04 (0.15)	1 (0.15)	1.02 (0.14)	1.08 (0.14)	1.06 (0.13)	1.07 (0.08)	0.97 (0.15)	0.93 (0.14)	0.97 (0.17)
Z-score, mean, (SD)									
LS	-0.52 (1.13)	-0.39 (1.15)	-0.23 (0.93)	-0.7 (1.15)	-0.35 (1.28)	-0.22 (1.19)	-0.26 (1.06)	-0.43 (1)	-0.24 (0.56)
FN	0.01 (1.02)	-0.06 (0.85)	0.26 (0.68)	-0.04 (1.02)	0.11 (0.89)	0.18 (0.6)	0.08 (1.04)	-0.27 (0.76)	0.34 (0.76)
TH	0.02 (1.08)	-0.18 (1.02)	0.11 (0.73)	0.05 (1.09)	0.07 (0.93)	0.04 (0.64)	-0.02 (1.09)	-0.48 (1.07)	0.2 (0.81)

Data are expressed as means and standard deviations for continuous, normally distributed variables. LS, lumbar spine; FN, femoral neck; TH, total hip

We wanted to compare both Z-scores and BMDs between the 3 groups depending on sex. We used MANOVA to evaluate the hypothesis that there is at least one difference between the groups. Firstly, the multivariate test, which protects our results against type I errors, showed a significant result (Pillai's Trace = 0.33, F(12, 164) = 2.74, p = 0.002 cu η^2 = 0.17). Further on, we performed a series of ANOVA tests to detect which groups in particular are different from each other and also a post-hoc analysis with Bonferroni corrections to identify the variables responsible for the differences.

The Z-scores at the femoral neck were significantly lower in HCV women than in control women (p = 0.02). The mean difference between these groups was 0.6 (95%CI = 0.07 - 1.14). Also, total hip Z-score was 0.68 lower (95%CI = 0.06 - 1.3) in HCV women *versus* control women. No other differences between the hepatitis groups and the control group, nor between HBV and HCV groups were found.

Correlations analysis between BMD measurements and anthropometric/disease-related characteristics

When HBV patients were included in the analysis, statistically significant negative correlations with age were found for both Z-scores and BMDs (g/cm^2) at lumbar spine and femoral neck. Similar results were found in the male HBV group, but not in the female group as shown in Table IV.

Table IV

Correlations between the age of HBV patients and their bone mineral densities (g/cm^2) and Z-scores

All patients (n = 66)						
	LS		**FN**		**TH**	
Age	**BMD**	**Z-score**	**BMD**	**Z-score**	**BMD**	**Z-score**
	ρ = -0.395	ρ = -0.441	ρ = -0.264	ρ = -0.248	ρ = -0.122	ρ = -0.112
	p = 0.001	**p < 0.001**	**p = 0.032**	**p = 0.045**	p = 0.33	p = 0.44

Males (n = 38)						
Age	LS		FN		TH	
	BMD	Z-score	BMD	Z-score	BMD	Z-score
	ρ = -0.57	ρ = -0.594	ρ = -0.417	ρ = -0.251	ρ = -0.338	ρ = -0.274
	p < 0.001	**p < 0.001**	**p = 0009**	**p = 0.031**	**p = 0.038**	p = 0.096
Females (n = 28)						
Age	LS		FN		TH	
	BMD	Z-score	BMD	Z-score	BMD	Z-score
	ρ = -0.159	ρ = -0.289	ρ = -0.181	ρ = -0.132	ρ = 0.085	ρ = -0.056
	p = 0.419	p = 0.135	p = 0.358	p = 0.505	p = 0.667	p = 0.776

The relationships between variables are estimated by Spearman's ρ correlation coefficient and significant associations (p≤0.05) are highlighted. LS, lumbar spine; FN, femoral neck; TH, total hip

We also found that in males with HBV, disease length negatively correlates with femoral neck Z-scores (ρ = -0.35, p = 0.03) and that previously treated patients had lower lumbar spine BMD than treatment-naïve ones (ρ = -0.35, p = 0.03), Figure 1. Fibrosis grade also correlated negatively with Z-score at lumbar spine (ρ = -0.26, p = 0.03). BMI and viral load did not correlate significantly with any of the bone mass variables in this group.

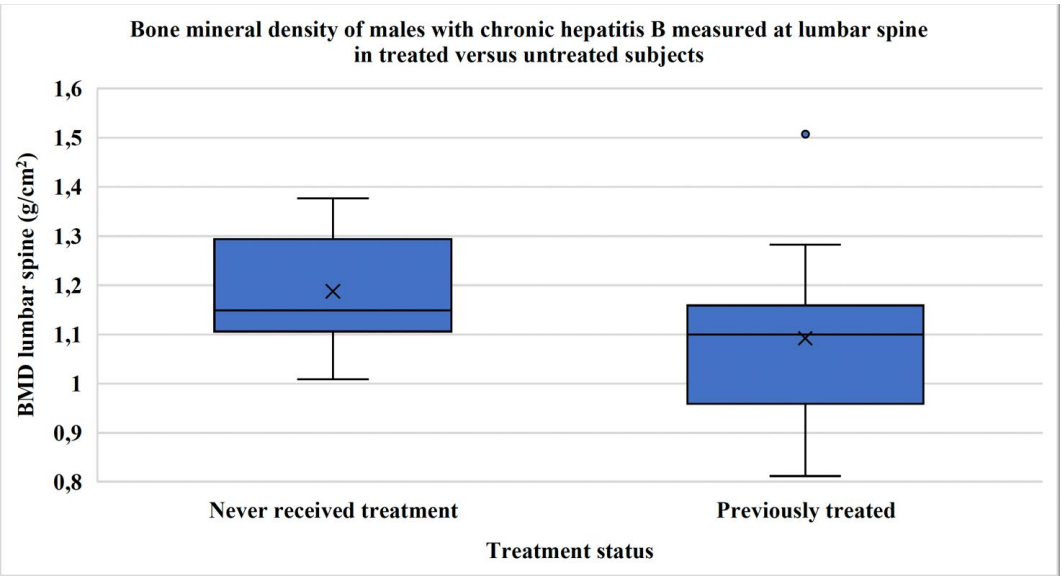

Figure 1.
Increased bone density of the lumbar spine in treatment-naïve patients with chronic hepatitis B *versus* patients that have received antiviral medication (p = 0.03)

In the hepatitis C group, BMI was positively correlated with BMDs (g/cm^2) at all skeletal sites: lumbar spine (ρ = 0.25, p = 0.04), femoral neck (ρ = 0.27, p = 0.03) and total hip (ρ = 0.37, p = 0.002); the correlations remained significant for males at lumbar spine and total hip (ρ = 0.38, p = 0.024, ρ = 0.34, p = 0.045 respectively) and females at femoral neck and total hip (ρ = 0.4, p = 0.03, ρ = 0.43, p = 0.016 respectively).

Unlike hepatitis B group, no significant correlations were found between bone mass variables and age when all patients were included in the analysis. Disease-related variables (fibrosis grade, disease length, viral load and treatment) did not significantly correlate with bone mineral density either.

We performed a multiple linear regression analysis to identify predictors for bone loss in HBV and HCV patients. The analysis for HBV patients is shown in Table V. In the hepatitis B group, we found models that may predict Z-scores at lumbar spine and femoral neck based on age and disease length – both correlated negatively. Z-score at lumbar spine would be equal to 1.17 – 0.64 (age category) – 0.04 (disease length) – 0.5 (fibrosis grade). Even though BMI was correlated with BMD at all sites, no model including the nutritional status as predictor proved significant.

Table V

Multivariable linear regression results for HBV group

		Z-score LS			Z-score FN		
		All patients	**Males**	**Females**	**All patients**	**Males**	**Females**
Age category		-0.376* (p = 0.02)	-0.474** (p = 0.002)	-0.398 (p = 0.112)	-0.284* (p = 0.021)	-0.413** (p = 0.009)	-0.179 (p = 0.409)
BMI		-0.123 (p = 0.279)	-0.218 (p = 0.12)	0.056 (p = 0.794)	-	-	-
Disease length		-0.229* (p = 0.037)	-0.244 (p = 0.079)	0.054 (p = 0.783)	-0.174 (p = 0.15)	-0.338* (p = 0.048)	0.062 (p = 0.771)
Viral load		-	-	-	-	-	-
Fibrosis grade		-0.156 (p = 0.163)	-0.09 (p = 0.514)	-0.265 (p = 0.199)	-	-	-
Treatment		-	-	-	-	-	-
R^2		0.328	0.436	0.276	0.131	0.294	0.047
Adjusted R^2		0.284	0.368	0.12	0.103	0.231	-0.072
Model adequacy	**F**	7.451**	6.384**	2.194	4.749*	4.709**	0.395
	P	< 0.001	< 0.001	0.192	0.012	0.007	0.758
Number of observations		66	38	28	66	38	28

Variables included in the models were selected from the correlation analyses. β coefficients are shown for each variable. The regression models were adjusted according to the F-test of the ANOVA. Significant results at 0.05 and 0.01 level are marked with * and ** respectively

Bone metabolism alterations are a frequent complication of liver diseases regardless of their aetiology [11, 18]. Osteoporosis and osteopenia are the most cited ones, while osteomalacia is a very rare complication, mostly associated with longstanding illnesses [10]. The prevalence varies largely among authors (with osteopenia prevalence ranging from 12% to 55%), but most results confirm increasing percentages in patients with increased disease duration and fibrosis severity [8].

In this study, we focused on a selected category of subjects: patients with chronic HBV/HBC infection that did not present a series of risk factors previously known to favour bone demineralization (i.e., advanced age, postmenopausal status, chronic diseases of either bone or other organs, medication associated with bone loss, malnutrition and immobility) and their risk of developing bone loss compared to the general population. The anthropometric characteristics of the 3 study groups were similar: the subjects were young – with a mean age of 36 years old and they had normal BMIs which enables an accurate comparison of the patients with healthy subjects.

The analysis of disease-related parameters showed significant differences between HBV and HCV groups. HCV patients had higher FibroTest scores, more frequently positive viral loads and a greater proportion of them received treatment compared to the HBV group. These differences could be explained by the strict criteria of inclusion in therapeutic protocols.

The prevalence of low BMD was found to be 11% in both hepatitis groups; this was found to be 5 times greater than the control group. The most affected sites were the lumbar spine for HBV patients and both the lumbar spine and hip for the HCV patients. Other studies that included non-cirrhotic patients with viral hepatitis had similar results: *Schiefke et al*. investigated 43 patients with HCV or HBV, mean age 49 years old and found osteoporosis in between 7% and 19% depending on the site [27]. Huang *et al*. identified an overall prevalence of 12.8% in their HBV cohort (mean age = 42 years old), but higher numbers were found when postmenopausal women and elderly were analysed separately [15]. In another research, Barbu *et al*. included non-cirrhotic patients of all ages and found even higher rates of demineralization (33%) [2]. Regarding the age of the investigated patients, even though our study included young subjects, we found negative correlations with age in the HBV group; the association was also reported by other authors [2, 15].

In patients with HIV infection, the presence of viral hepatitis co-infections has shown a significant decrease in bone mineral density [21]. Negative associations were also found between Z-scores at femoral neck and disease length as well as BMD at lumbar spine and the history of treatment. One of the antivirals used in HBV protocols, tenofovir disoproxil fumarate (TDF), is well known for his negative impact on bone metabolism and certain guidelines decided to recommend a replacement with tenofovir alafenamide (TAF) in high-risk individuals [10, 21–23]. Its effects were more thoroughly studied in HIV cohorts where patients are at particular risk for bone diseases considering the HIV infection itself and the long-term antiretroviral therapy (ART) [4, 5, 7]. Moreover, HIV/viral hepatitis co-infected patients seem to have even more pronounced bone loss – especially, women as reported by Lo Re *et al*. – but the pathogenesis behind this relationship is not well understood [24]. Regarding the history of treatment, in this research the patients with chronic HBV and also HCV infection followed different and multiple treatment regimens, according to treatment protocols. Given the multiple regimens that some patients received during the course of the disease, as

well as the lack of treatment in other patients who had no indication for treatment, we chose to divide patients according to this criterion (untreated *versus* previously treated).

In the studied HCV group, positive correlations were found between BMIs and BMDs at all three sites investigated. Other studies also confirmed this relationship [2, 3, 14]. Several hypotheses have been stated in order to explain the protective role of the adipose tissue on bone mass: greater oestrogen levels, increased mechanical load that promotes bone synthesis, the secretion of certain cytokines such as leptin and adiponectin [9, 29, 32]. However, epidemiological data show that obese subjects have increased risk of fractures despite their higher BMDs, which should prompt clinicians to screen and treat these patients earlier in the course of the disease [9].

We believe that reactivation of viral hepatitis may accelerate demineralization, especially in condition of nonresponse to viral therapy, but till this moment we do not find any research able to offer an answer. In a recent research, patients with reactivation of hepatitis B in condition of chemo-immunotherapy for haematological malignancies were treated with entecavir and they had a favourable outcome [19], and that may slow the demineralization rate.

Toxic hepatitis may also have a role in bone demineralization, especially if some drugs that are administrated in a period large enough so that the demineralization may occur. Many drugs may interfere with demineralization mechanisms, including gluco-corticoids and hepatitis antiviral therapy. It is possible that even antituberculosis therapy that may cause toxic hepatitis may interfere with bone mass loss, as the period of treatment is long, especially in nonresponsive patients [1, 11].

Some limitations were noted in this study. Firstly, the design of the study doesn't allow for evaluation of the causal relationship between viral hepatitis and low BMD – a longitudinal design would be more adequate for this purpose. The patients were enrolled in a specialized centre for infectious diseases and they may not accurately represent all the HBV/HCV patients. Bone metabolism was only evaluated by DXA, but serum markers could be also used in order to refine these results. And lastly, more behavioural patterns could be investigated in the future – both preventive (physical activity, balanced nutrition) and risky (smoking, alcohol consumption).

Conclusions

In this particular group of chronic viral hepatitis patients – non-cirrhotic, young/premenopausal and without comorbidities – few studies have described the risk for low BMD and the predisposing or protective factors.

In our research, patients with chronic viral hepatitis B or C acquired reduced bone mineral density more frequently than the control group, leading to secondary demineralization. The most affected sites were the lumbar spine for the hepatitis B patients and, both the lumbar spine and hip for the hepatitis C patients, respectively. We identified several risk factors that correlated with reduced bone mass: age (in hepatitis B patients), gender (in both hepatitis B and C patients), body mass index (hepatitis C patients), fibrosis grade (hepatitis B patients), length of the disease (femoral neck demineralization in males with hepatitis B) and history of antiviral treatment (lumbar spine demineralization in males with hepatitis B). One of the most important results is that males with hepatitis B were more affected by reduced bone mineral density than females; also, men with hepatitis B showed demineralization in the femoral head as the disease progresses and de-mineralization in the lumbar spine if previously treated. Thus, our results are of great significance to the medical and research community and they will be conductive to more efficient prevention and treatment for this complication as well better long-term health-related quality of life for these young, active patients.

Conflict of interest

The authors declare no conflict of interest.

References

1. Barbu EC, Chițu-Tișu CE, Lazăr M, Olariu C, Bojincă M, Ionescu RA, Ion DA, Bădărău IA, Hepatic osteodystrophy: A global (re)view of the problem. *Acta Clin Croat.*, 2017; 56(3): 512-525.
2. Barbu EC, Chițu-Tișu CE, Lazăr M, Olariu CM, Olteanu D, Bojincă M, Abagiu AO, Aramă V, Ion DA, Bădărău IA, Body composition changes in patients with chronic hepatitis C. *J Gastrointest Liver Dis.*, 2016; 25(3): 323-329.
3. Bering T, Diniz KGD, Coelho MPP, Vieira DA, Soares MMS, Kakehasi AM, Correia MITD, Teixeira R, Queiroz DMM, Rocha GA, Silva LD, Association between pre-sarcopenia, sarcopenia, and bone mineral density in patients with chronic hepatitis C. *J Cachexia Sarcopenia Muscle*, 2018; 9(2): 255-268.
4. Chițu-Tișu CE, Barbu EC, Lazăr M, Ionescu R, Bojinca M, Ion DA, Badarau IA, An overview of bone disease in HIV-infected patients. *Acta Medica Mediterr.*, 2015; 31(6): 1139-1151.
5. Chițu-Tișu CE, Barbu EC, Lazăr M, Bojincă M, Tudor AM, Hristea A, Abagiu AO, Ion DA, Bădărău AI, Body composition in HIV-infected patients receiving highly active antiretroviral therapy. *Acta Clin Belg.*, 2017; 72(1): 55-62.
6. Collier J, Bone disorders in chronic liver disease. *Hepatology*, 2007; 46(4): 1271-1278.
7. Compston J, HIV infection and osteoporosis. *Bonekey Rep.*, 2015; 4: 636: 1-5.
8. Ehnert S, Aspera-Werz RH, Ruoß M, Dooley S, Hengstler JG, Nadalin S, Relja B, Badke A, Nussler AK, Hepatic Osteodystrophy-Molecular Mechanisms

Proposed to Favor Its Development. *Int J Mol Sci.*, 2019; 20(10): 2555: 1-31.
9. Fassio A, Idolazzi L, Rossini M, Gatti D, Adami G, Giollo A, Viapiana O, The obesity paradox and osteoporosis. *Eat Weight Disord.*, 2018; 23(3): 293-302.
10. Gatta A, Verardo A, Di Pascoli M, Giannini S, Bolognesi M, Hepatic osteodystrophy. *Clin Cases Miner Bone Metab.*, 2014; 11(3): 185-191.
11. Garlitska N, Fira L, Lykhatskyi P, Boyko L. Biochemical mechanisms of oxidative stress in animals exposed to hexavalent chromium compounds in the case of isoniazid–rifampicin hepatitis. *Farmacia*, 2021; 69(2): 253-259.
12. González-Calvin JL, Mundi JL, Casado-Caballero FJ, Abadia AC, Martin-Ibañez JJ, Bone mineral density and serum levels of soluble tumor necrosis factors, estradiol, and osteoprotegerin in postmenopausal women with cirrhosis after viral hepatitis. *J Clin Endocrinol Metab.*, 2009; 94(12): 4844-4850.
13. Grigsby IF, Pham L, Mansky LM, Gopalakrishnan R, Mansky KC, Tenofovir-associated bone density loss. *Ther Clin Risk Manag.*, 2010; 6(1): 41-47.
14. Hofmann WP, Kronenberger B, Bojunga J, Stamm B, Herrmann E, Bücker A, Mihm U, von Wagner M, Zeuzem S, Sarrazin C, Prospective study of bone mineral density and metabolism in patients with chronic hepatitis C during pegylated interferon alpha and ribavirin therapy. *J Viral Hepat.*, 2008; 15(11): 790-796.
15. Huang Z, Wei H, Cheng C, Yang S, Wang J, Liu X, Low bone mineral density in chronic hepatitis B virus infection: A case-control study. *Pakistan J Med Sci.*, 2017; 33(2): 457-461.
16. Inaam A Nakchbandi, Schalk W van der Merwe, Current understanding of osteoporosis associated with liver disease. *Nat Rev Gastroenterol Hepatol.*, 2009; 6(11): 660-670.
17. Jeong HM, Kim DJ, Bone diseases in patients with chronic liver disease. *Int J Mol Sci.*, 2019; 20(17): 4270: 1-27.
18. Lazzaro L, Tonkin BA, Poulton IJ, McGregor NE, Ferlin W, Sims NA, IL-6 trans-signalling mediates trabecular, but not cortical, bone loss after ovariectomy. *Bone*, 2018; 112: 120-127.
19. Molagic V, Tilișcan C, Popescu C, Aramă V, Rădulescu M, Vlădăreanu AM, Aramă ȘS. Hepatitis B virus reactivation in patients undergoing chemoimmunotherapy for haematological malignancies. *Farmacia*, 2020; 68(2): 256-260.
20. Mysore KR, Leung DH, Hepatitis B and C. *Clin Liver Dis.*, 2018; 22(4): 703-722.
21. Negru A, Tilișcan C, Tudor AM, Oprea C, Ianache I, Lazăr M, Munteanu D, Mardarescu M, Streinu-Cercel A, Aramă V, Aramă SS, Identifying the risk factors for low bone mineral density disorders in HIV-positive patients undergoing antiretroviral therapy from the Romanian cohort. *Farmacia*, 2019; 67(3): 383-391.
22. Nielsen KO, Mirza AH, Kaur S, Jacobsen KS, Winther TN, Glebe D, Pociot F, Hogh B, Størling J, Hepatitis B virus suppresses the secretion of insulin-like growth factor binding protein 1 to facilitate anti-apoptotic IGF-1 effects in HepG2 cells. *Exp Cell Res.*, 2018; 370(2): 399-408.
23. Raslan HM, Elhosary Y, Ezzat WM, Rasheed EA, Rasheed MA, The potential role of insulin-like growth factor 1, insulin-like growth factor binding protein 3 and bone mineral density in patients with chronic hepatitis C virus in Cairo, Egypt. *Trans R Soc Trop Med Hyg.*, 2010; 104(6): 429-432.
24. Lo Re V, Guaraldi G, Leonard MB, Localio AR, Lin J, Orlando G, Zirilli L, Rochira V, Kostman JR, Tebas P, Viral Hepatitis is Associated with Reduced Bone Mineral Density in HIV-Infected Women But Not Men. *AIDS*, 2009; 23(16): 2191-2198.
25. Roade L, Riveiro-Barciela M, Esteban R, Buti M, Long-term efficacy and safety of nucleos(t)ides analogues in patients with chronic hepatitis B. *Ther Adv Infect Dis.*, 2021; 8: 2049936120985954: 1-13.
26. Orsini LG, Pinheiro MM, Castro CH, Silva AE, Szejnfeld VL, Bone Mineral Density Measurements, Bone Markers and Serum Vitamin D Concentrations in Men with Chronic Non-Cirrhotic Untreated Hepatitis C. *PLoS One*, 2013; 8(11): e81652: 1-5.
27. Schiefke I, Fach A, Wiedmann M, Aretin AV, Schenker E, Borte G, Wiese M, Moessner J, Reduced bone mineral density and altered bone turnover markers in patients with non-cirrhotic chronic hepatitis B or C infection. *World J Gastroenterol.*, 2005; 11(12): 1843-1847.
28. Schousboe JT, Shepherd JA, Bilezikian JP, Baim S, Executive summary of the 2013 International Society for Clinical Densitometry Position Development Conference on bone densitometry. *J Clin Densitom.*, 2013; 16(4): 455-466.
29. Shen J, Nielson CM, Marshall LM, Lee DC, Keaveny TM, Orwoll ES; Osteoporotic Fractures in Men MrOS Research Group, The Association Between BMI and QCT-Derived Proximal Hip Structure and Strength in Older Men: A Cross-Sectional Study. *J Bone Miner Res.*, 2015; 30(7): 1301-1308.
30. Sudjaritruk T, Bunupuradah T, Aurpibul L, Kosalaraksa P, Kurniati N, Sophonphan J, Ananworanich J, Bone-D study group, Impact of tenofovir disoproxil fumarate on bone metabolism and bone mass among perinatally HIV-infected Asian adolescents. *Antivir Ther.*, 2017; 22(6): 471-479.
31. World Health Organizastion, Body mass index – BMI. 2021.
32. Zhao LJ, Jiang H, Papasian CJ, Maulik D, Drees B, Hamilton J, Deng HW, Correlation of Obesity and Osteoporosis: Effect of Fat Mass on the Determination of Osteoporosis. *J Bone Miner Res.*, 2008; 23(1): 17-29.