

## Osteoporosis in Chronic Hepatitis C Virus with Advanced Liver Fibrosis

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Received: October 23, 2014

Revised: November 9, 2014

Accepted: November 15, 2014

Published online: December 21, 2014

**CONCLUSIONS:** Our study showed an association between advanced stage of liver fibrosis, the level of viremia and decreased BMD in chronic HCV patients. Replacement therapy for osteoporosis in advanced liver fibrosis caused by HCV is recommended.

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**Key words:** BMD; DXA; Osteoporosis; Smoking; Advanced liver fibrosis and chronic HCV

El-Atrebi KA, El-Bassyouni HT, El-AtrebiAA. Osteoporosis in Chronic Hepatitis C Virus with Advanced Liver Fibrosis. *Journal of Gastroenterology and Hepatology Research* 2014; 3(12): 1392-1395 Available from: URL: <http://www.ghrnet.org/index.php/joghr/article/view/968>

### ABSTRACT

**AIM:** The incidence of osteoporosis in chronic liver diseases varies widely, and is dependent on the severity of the underlying liver disease. The aim of this work was to evaluate the bone mineral density (BMD) in chronic hepatitis C virus (HCV) patients and explore the association between the degree of liver disease and BMD.

**METHODS:** Patients ( $n=88$ ) were recruited from the Hepatology Department, National Hepatology and Tropical Medicine Research Institute, Egypt and dual-energy X-ray absorptiometry (DXA) was used for the determination of the BMD. Liver biopsy was performed to evaluate fibrosis staging.

**RESULTS:** The extent of osteoporosis was greater in patients with advanced fibrosis as compared to patients with early stage of fibrosis. There was also significant correlation between fibrosis stage and BMD. Smoking significantly associated with osteoporosis. When smoking was added as a cofactor, univariate analysis by logistic regression found that the presence of advanced fibrosis was associated with decreased BMD.

### INTRODUCTION

Osteoporosis is a disease characterized by systemic loss of bone mineral and is considered to be one of the major public health problems today<sup>[1]</sup>. Hepatitis C is a global health problem caused by infection with the hepatitis C virus (HCV); it is estimated that as many as 170 million persons worldwide may be infected with HCV<sup>[2]</sup>. The term "hepatic osteodystrophy" encompasses bone disease associated with chronic liver disease and includes osteopenia/osteoporosis and, more rarely, osteomalacia<sup>[3,4]</sup>. In addition to severe cholestatic liver disease, significant osteoporosis is generally reported in patients with cirrhosis, especially secondary to hepatitis C<sup>[4-6]</sup>. Cirrhotic patients with viral hepatitis display a prevalence of osteoporosis up to 53%<sup>[7]</sup>. The etiology of these disorders is complex and multifactorial<sup>[3,5]</sup>. The incidence of osteoporosis in chronic liver diseases varies widely, depending on the patient population and the underlying liver disease and its severity<sup>[4]</sup>. Only a few studies on bone formation have been performed in patients suffering from chronic hepatitis C (HCV)<sup>[7]</sup>. Most studies of bone disease were performed on patients with cirrhosis. Nevertheless, little is known about the

occurrence of bone disease in non-cirrhotic patients with chronic hepatitis C<sup>[8]</sup>. The aim of the work is to evaluate the bone mineral density (BMD) in chronic hepatitis C virus (HCV) patients to unravel the association between the degree of liver disease and BMD in chronic hepatitis C virus patients.

## METHODS

Eighty-eight patients of the outpatient department (Hepatology and Tropical Medicine Research Institute) with chronic HCV infection participated in this study. The group consisted of 76 men and 12 young women (menstruating).

Full history taking including smoking and drug history of drugs that could affect the bone marrow metabolism such as hormone replacement therapy, corticosteroid, proton pump inhibitors, calcineurin inhibitor, loop diuretics, heparin, anti-coagulant, selective inhibitors of serotonin reuptake, antiretroviral and meticulous clinical examination were done for all patients. Selected patients had positive serological markers and a positive polymerase chain reaction (PCR) for HCV infection for more than 6 months.

The relevant demographic- and disease-related data at the time of enrollment were listed in (Table 1). Decompensated cirrhotic patients were excluded, as all patients were subjected to a liver biopsy within 6 months prior to study entry, abdominal ultrasonography, and laboratory findings. The exclusion criteria were presence of hepatocellular carcinoma; human immunodeficiency virus (HIV) coinfection; hepatitis B virus coinfection; autoimmune liver disease defined according to validated diagnostic criteria<sup>[9]</sup> and genetic liver disease (e.g., Wilson disease, hemochromatosis). After taking informed consent, liver biopsy was performed for the 44 patients using 16-gauge true-cut needles and was scored using Metavir scoring system. According to the Metavir system, fibrosis was staged on a scale from F0 to F4; F0-no fibrosis; F1-portal fibrosis without septa; F2-few septa; F3-many septa without cirrhosis and F4-cirrhosis or advanced scarring of the liver. The F0 and F1 were considered minimal or no fibrosis, whereas the scores F2-F4 were considered advanced fibrosis<sup>[10]</sup>. Data about body weight and height, BMI, physical activity, menstrual status, number of pregnancies, and amount of coffee intake were obtained. None of the patients had a family history of osteoporosis, nor did they receive calcium, vitamin D, corticosteroids, or any medication related to mineral metabolism. All patients signed informed consent and the protocol was approved by the local human ethical committee.

### Bone densitometry

The 88 patients underwent densitometric evaluation with a Lunar Prodigy Advance device. Two kinds of explorations following standard criteria were performed: a specific bone densitometric study of hip and lumbar spine, in order to record Z and T scores<sup>[11]</sup>. Because there exists the possibility of ambiguity when attempting to achieve accuracy within dual-energy X-ray absorptiometry (DXA)<sup>[12,13]</sup>, we used a simple method of body mass index (BMI) to assess body composition. BMI was defined as weight (kilograms) divided by height (meters) squared. A wall-mounted stadiometer was used to measure the height. Each participant was weighed while wearing minimal clothing. All patients underwent DXA scans to determine the BMD in the lumbar spine (L1-L4) and the left femoral neck. A single technician certified by the International Society of Clinical Densitometry performed all DXA scans on a fan-beam bone densitometer (QDR-4500A; Hologic, Waltham, MA). DXA data for age- and sex-matched controls were obtained from

**Table 1** Basic characteristic of the studied patients.

|                             | Mean ± SD               | Range                  |
|-----------------------------|-------------------------|------------------------|
| <b>Demographic features</b> | Age (years)             | 44±10                  |
|                             | BMI                     | 27.05±4.5              |
|                             | Gender                  | 12 Females<br>76 Males |
|                             | Smokers                 | 32                     |
|                             | PCR (IU/mL)             | Median (IQR)           |
| <b>Laboratory results</b>   | Calcium (mg/dL)         | 106100 (715267)        |
|                             | Phosphate (IU/L)        | 9.4±0.64               |
|                             | ALP (U/mL)              | 3.50±0.42              |
|                             | Serum bilirubin (mg/dL) | 146.41±90              |
|                             | Serum albumin (g/dL)    | 0.76±0.18              |
| <b>Liver histopathology</b> | INR                     | 4.2±0.25               |
|                             | Fibrosis staging        | 1.01±0.5               |
|                             | advanced (72)           | 81.8 %                 |
|                             | early (12)              | 13.6 %                 |
|                             | No fibrosis (4)         | 4.5 %                  |
| <b>DXA (Qualitative)</b>    | Normal (52)             | 59.1 %                 |
|                             | Osteopenia (20)         | 22.7 %                 |
|                             | Osteoporosis (16)       | 18.2 %                 |
| <b>DXA (Quantitative)</b>   | T score                 | Median (IQR)           |
|                             | T score N(neck femur)   | -0.135 (1.9)           |
|                             | Z score                 | -0.500 (1.4)           |
|                             | Z score N(neck femur)   | -0.28 (2)              |
|                             |                         | Range                  |
|                             |                         | -3.4 -2.5              |
|                             |                         | -3.0 - 1.0             |
|                             |                         | -2.0 - 3.0             |

IQR: Inter Quartile Range.

the DXA manufacturer's reference population and comprised the BMD reference in this study. For descriptive analyses, osteopenia was defined as T score between -1.0 and -2.5 while osteoporosis was defined as a T score < -2.5.

### Statistical methods

Qualitative data were presented by number and percent. They were compared by Chi-square or Fischer's exact test when appropriate. While quantitative data were presented by mean and standard deviation for parametric data, and by median and interquartile range for non-parametric data. They were compared by t-student or Mann Whitney U test respectively. Univariate logistic regression analysis was done in which the abnormality of DXA scan was the dependent factor, age, gender, BMI, PCR, Calcium, Phosphorous, Alkaline phosphatase, smoking and fibrosis staging were the independent factors. In all tests, the *P* value was considered significant if less than 0.05.

## RESULTS

The demographic and clinical data at the time of enrollment are listed in (Table 1). The mean age of the 88 patients included in the study was 44 years ranged from 22-59 years and the male to female ratio was 6.3:1.4. The mean BMI of the studied patients was 27.05±4.5 (19-38) and 36.4 % of them were smokers.

The quantitative DXA results were presented in the median inter quartile range (IQR): total T scores were -0.135 (1.9), the femur neck (FN) region was -0.500 (1.4). The mean Z score was -0.28 (2) (-3-1) and the FN region was 0.38 (2). Moreover, the qualitative DXA results were: 20 (22.7%) patients had osteopenia, 16 (18.2%) patients had osteoporosis and 52 (59.1%) patients were normal.

The mean±SD values of the biochemical findings were as follows: Total Calcium 9.4±0.64, phosphorous 3.5±0.42 and alkaline phosphatase 146.41±90. Liver synthetic functions were: serum bilirubin 0.76±0.18, serum albumin 4.2±0.25, international normalization ratio (INR) 1.01±0.5 and the PCR median (IQR) was 106,100 (715,267) IU/ML.

Liver histopathology showed high staging (F3-4) in 72 patients

(81.8%), low staging (F1-2) in 12 patients (13.6%) and no fibrosis was detected in 4 patients (4.5%).

Comparing the patients in relation to their DXA scan (Table 2) showed significant decrease in BMD in older HCV patients and also in cases of hypocalcaemia.

The fibrosis stage was significantly higher in patients having high BMI. The T-score was much less in patients with advanced fibrosis. Serum calcium was significantly higher in patients with early fibrosis stage (Table 3).

The relationship between fibrosis stage and BMD showed significant association, as osteopenia and osteoporosis were associated with advanced fibrosis (Table 4).

Patients' data and smoking habits relationship showed significant association between smoking and older age, higher baseline HCV viremia and lower T and Z scores, moreover smoking and osteoporosis were significantly associated (Table 5).

## DISCUSSION

Osteoporosis is a frequent complication of end-stage liver disease. The prevalence varies between 9% and 60%, the highest being observed in cholestatic disorders and alcoholic liver disease<sup>[8]</sup>. Cirrhosis is generally associated with lower BMD as it shows reduction in osteoid

**Table 2** The relation between the patient's data and DXA results.

|                             | Normal DXA |            | Normal DXA |            | p value            |
|-----------------------------|------------|------------|------------|------------|--------------------|
|                             | Mean       | SD         | Mean       | SD         |                    |
| Age                         | 40.00      | 9.466      | 40.00      | 9.466      | 0.001 <sup>2</sup> |
| BMI                         | 27.46      | 4.356      | 27.46      | 4.356      | 0.470              |
| PCR (IU/mL)                 | 279399.54  | 402410.273 | 279399.54  | 402410.273 | 0.056 <sup>1</sup> |
| Calcium (mg/dL)             | 9.638      | 0.5193     | 9.638      | 0.5193     | 0.002 <sup>2</sup> |
| Phosphate (IU/L)            | 3.515      | 0.2738     | 3.515      | 0.2738     | 0.839              |
| Alkaline phosphatase (U/mL) | 124.538    | 9.9166     | 124.538    | 9.9166     | 0.118              |

<sup>1</sup>Mann-Whitney Test, <sup>2</sup>Significant (P value ≤0.05).

**Table 3** The relation between the patient's data and fibrosis stage.

|                                     | No or early fibrosis |         | Advanced fibrosis |         | p value            |
|-------------------------------------|----------------------|---------|-------------------|---------|--------------------|
|                                     | Mean                 | SD      | Mean              | SD      |                    |
| Age                                 | 39.00                | 6.845   | 45.11             | 9.965   | 0.108              |
| BMI                                 | 31.00                | 5.071   | 26.17             | 3.961   | 0.005 <sup>2</sup> |
| <sup>1</sup> PCR (IU/mL)            | 62200                | 851800  | 104718            | 453598  | 0.363              |
| <sup>1</sup> T score                | 0.300                | 1.3     | -0.350            | 2       | 0.35               |
| <sup>1</sup> T score N (neck femur) | 0.000                | 3.1     | -0.650            | 1.6     | 0.019 <sup>2</sup> |
| <sup>1</sup> Z score                | -0.60                | 1       | -0.23             | 2       | 0.196              |
| <sup>1</sup> Z score N (neck femur) | -0.10                | 3       | 0.38              | 4       | 0.018 <sup>2</sup> |
| Calcium                             | 9.875                | 0.3655  | 9.294             | 0.6418  | 0.467              |
| Phosphate                           | 3.550                | 0.2673  | 3.494             | 0.4465  |                    |
| Alkaline phosphatase                | 125.000              | 12.6265 | 151.167           | 99.7341 |                    |

<sup>1</sup>Mann-Whitney Test, data presented by median (IQR),

<sup>2</sup>Significant (P value ≤0.05).

**Table 4** The relation between fibrosis stage and DXA results.

|       |          | Fibrosis          |          |        |        |
|-------|----------|-------------------|----------|--------|--------|
|       |          | No or early       | Advanced | Total  |        |
| DXA   | Normal   | Count             | 16       | 36     | 52     |
|       |          | % within fibrosis | 100.0%   | 50.0%  | 59.1%  |
|       | Abnormal | Count             | 0        | 36     | 36     |
|       |          | % within fibrosis | 0.0%     | 50.0%  | 40.9%  |
| Total |          | Count             | 16       | 72     | 88     |
|       |          | % within fibrosis | 100.0%   | 100.0% | 100.0% |

There is significant association between DXA results and fibrosis stage. Abnormal DXA results associated with advanced fibrosis (P=0.01).

**Table 5** The relations between the patient's data and smoking habits.

|              | No smoking             |        | Smoking |        | p value            |                    |
|--------------|------------------------|--------|---------|--------|--------------------|--------------------|
|              | Mean                   | SD     | Mean    | SD     |                    |                    |
| DEXA scores  | <sup>1</sup> T score   | 0.300  | 1.7     | -0.650 | 2.6                | 0.187              |
|              | <sup>1</sup> T score N | -0.200 | 2.2     | -0.880 | 1.9                | <0.01 <sup>2</sup> |
|              | <sup>1</sup> Z score   | -0.28  | 2       | -0.85  | 3                  | 0.06               |
|              | <sup>1</sup> Z score N | 0.50   | 2       | -0.27  | 2                  | 0.02 <sup>2</sup>  |
| DEXA results | No                     | %      | No      | %      | 0.027 <sup>2</sup> |                    |
|              | Normal                 | 40     | 71.4    | 12     |                    | 37.5               |
|              | Osteopenia             | 12     | 21.4    | 8      |                    | 25.0               |
|              | Osteoporosis           | 4      | 7.1%    | 12     |                    | 37.5               |

<sup>1</sup>Mann-Whitney Test, data presented by median (IQR),

<sup>2</sup>Significant (P value ≤0.05).

thickness, osteoblast surface and bone formation rate<sup>[8]</sup>.

Metabolic bone disease occurring in individuals with chronic liver disease, particularly in those with cholestasis, is known as hepatic osteodystrophy, whereas the relation between reduced BMD and noncirrhotic CHC patients who are non cholestatic or hypogonadal is still a matter of debate<sup>[1,13-15]</sup>.

In the present study the mean age was 44 years, the females were in the child-bearing period Joseph *et al*<sup>[16]</sup> admitted that decrease in bone density will be after the age of 50 in studying both males and females. Lucaci *et al*<sup>[17]</sup> and Schiefke *et al*<sup>[8]</sup> proposed that BMD is lower in the more advanced stages of liver disease (P=0.027). In addition, they suggested a statistically significant difference between early (staging 0-2) and advanced fibrosis (staging 3-4), and concluded a high prevalence of osteopenia and osteoporosis in patients with non-cirrhotic viral hepatitis; this coincides with our results. Furthermore, Lin *et al*<sup>[11]</sup> reported higher rates of osteopenia and osteoporosis in chronic HCV (Child-Pugh≤5) compared to the control group. Duarte *et al*<sup>[18]</sup> found a statistically significant difference between the early and advanced stages of liver fibrosis, as BMD of the femoral neck (p=0.009) or lumbar spine (p=0.011) was lower in advanced fibrosis. These results are in accordance with our results, that showed a correlation between osteoporosis and the advanced affection of the liver. In contrast Pelazas-González *et al*<sup>[19]</sup> did not find correlation between bone variables and liver fibrosis. This may be because most of their studied patients had mild fibrosis. Furthermore, Luchi *et al*<sup>[20]</sup> reviewed 60 HCV patients, 34 of them had osteopenia, and 7 had osteoporosis. Schiefke *et al*<sup>[8]</sup> documented that HCV patients had osteoporosis affecting 7% at the spine and 19% at the hip. Moreover, they realized that the more the liver affection the more the osteoporosis and patients with early stages of fibrosis had near normal Z-scores. Ricard Blum *et al*<sup>[21]</sup> stated that increased telopeptide levels indicated increased bone breakdown and may also be related to liver fibrosis. Furthermore, Gallego-Rojo *et al*<sup>[7]</sup> delineated that patients with HCV showed decreased IGF-1 values which correlated with BMD.

In our study we found normal calcium level and statistical significant difference between both groups early fibrosis versus advanced fibrosis. Moreover, we found significant negative correlation between the level of viremia and the BMD, this was slightly different than Lucaci *et al*<sup>[17]</sup> who did not find correlation but the odd ratio of viral load was more than 1. In our study BMI was significantly higher in the group of no or early liver fibrosis, similarly Ydreborg *et al*<sup>[22]</sup> stated that BMI has been consistently associated with fibrosis.

In our study osteoporosis significantly associated with smoking. Recently, studies have shown a direct relationship between tobacco use and decreased bone density. Nevertheless, analyzing the impact of cigarette smoking on bone health is complicated. So, it is hard to determine whether the decrease in bone density is due to smoking itself or to other risk factors common among smokers<sup>[23-25]</sup>. Therefore,

we added smoking as a cofactor in logistic regression and proved that advanced fibrosis associated with abnormal BMD in a univariate analysis. On the contrary Pelazas-González *et al.*<sup>[19]</sup> did not find a significant associations between either BMD and smoking nor with fibrosis. One limitation of this study was the amount difference between both groups (early or no fibrosis and advanced fibrosis) this was because of the randomization regarding the stage of fibrosis.

## CONCLUSION

Our study showed an association between advanced stage of liver fibrosis, high viral load and decreased BMD in chronic HCV patients. Replacement therapy for osteoporosis in advanced liver fibrosis caused by HCV is recommended.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interests and received no financial support.

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