Assessment of Bone Density and Vit D. In Chronic Hepatitis C Infection

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Abstract: Aim of the Work: aim of this study was to assess bone mineral density and estimation of Vit. D status in patients with chronic hepatitis C viral infection. **Patients and Methods:** The study included 50 patients chronic hepatitis C (25 males and 25 females) All patients had positive test for anti-HCV antibody for at least 6 months and HCV-RNA detectable in the serum and 50 matched controls (25 males and 25 females). All participants were subjected to: Assessment of bone mineral density (g/cm^2) (BMD) by the dual energy X-ray absorptiometry method. And Serum 25 $(OH)D_3$ levels were measured by Chemoillumicience technique. Serum25 (OH)-D levels at 30 and 20 ng/ml were the cut off values for VD insufficiency and deficiency respectively. Both BMD **(g/cm^2),** and Serum25 (OH)-D were correlated with staging of liver disease choronicity as assessed by the Child-Pugh's score (CPS), gender age and disease duration were considered. **Results:** Bone mineral density were significantly lower in patients than controls (where $P = 0.078$ & 0.068), females patents were lower than males (non significant where $p < 0.05$) and vit D(OH) were also lower in patents (28.7 ± 5.4 ng/ml than controls 48± 67 ng/mL) (where $p<0.05$). **Conclusion:** BMD (osteopenia and osteoporosis) was more in CLD patients than controls and equal in both males and female patients, and correlated to disease duration and staging of liver disease. Vitamin D insufficiency and deficiency are common among chronic hepatitis C infection patients than controls also female patients had low vit. D (deficiency) than males.

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Introduction:

Infection with hepatitis C virus (HCV) is a major health problem and is one of the most important causes of chronic liver diseases. According to the World Health Organization (WHO) at least 170 million people are infected worldwide with HCV and 3 to 4 million new infections occur per year **(**Metts et al., 2014, Averhof et al., 2012) [8&10].

Chronic hepatitis C infection is defined as the presence of detectable viral replication for at least six months.

Today, HCV infection and its complications are among the leading public health challenges in Egypt (Saraswat et al., 2015) [1]. Egypt has the highest prevalence of HCV infection; about 9% country wide and up to 50% in certain rural areas Mueller et al., 2009 & (Guichelaar et al., 2006)[2&3]. Metabolic bone disease (often known as hepatic osteodystrophy (OH) is a common complication of long-standing liver disease (affects 12-55% of patients), and has an important effect on life as it causes fractures with immobilization (Guichelaar et. al 2006) [3].

Its etiology is not completely understood and it is thought to vary according to the type, severity and progression of the liver disease, along with a numerous of other contributing factors that influence bone formation or bone resorbtion (Lin et al., 2012)[4]. The effect of HCV exposure on bone mineral density in the absence of advanced liver disease remains debated. Some scholars have proposed that chronic HCV infection without liver cirrhosis contributes to reduced bone mineral density, whereas other scholars have asserted the opposite. (Lin et al 2013 & Hansen et. al., 2014) [4&30].

Vitamin D insufficiency and deficiency are prevalent in almost half the healthy population of developed countries Wariaghli et al., 2010[5]**.** Vitamin D is a fat soluble vitamin. It is a steroid hormone with pleitropic effects other roles in the body including modulation of cell growth, and reduction of inflammation. (Abu-Mouch et al., 11)[25].

Vitamin D is important in calcium homeostasis and has also been implicated in the mechanisms of cellular proliferation, differentiation, neuromuscular, reduction of inflammation, and immunomodulation (Bikle, 2009, & El-Habashy et al.14)[9&11]. These effects are noted in the pathogenesis and treatment of

many chronic liver diseases. (Backstedt et al., 2017)[9].

Vitamin D is obtained from dietary sources and through the photochemical conversion of 7-dehydrocholesterol in the **skin.** It binds to vitamin D-binding protein and is transported to the liver, where it is hydroxylated by 25-hydroxylases to form 25(OH)D, a stable metabolite that is the best single indicator of vitamin D status. A second hydroxylation step, mediated by the 1a-hydroxylase CYP27B1 in the kidneys and other extrarenal tissues, produces the most active metabolite $1,25(OH)_2D$, which signals primarily through the VDR, resulting in pleiotropic physiological effects,,$25(OH)_2D$ is catabolized by CPY24A1 to its inactive metabolite, calcitroic acid. (Villar et al., 2013, Terrier et al., 11) [26&27].

The role of vitamin D in the pathogenesis of NAFLD and CHC is not completely known, but it seems that the involvement of vitamin D in the activation and regulation of both innate and adaptive immune systems and its antiproliferative effect may explain its importance in these liver diseases. Published studies provide evidence for routine screening for hypovitaminosis D in patients with liver disease. Further prospectives studies demonstrating the impact of vitamin D replacement in NAFLD and CHC are required. (Mett, et al., 214) [8].

2. Patients and Methods

Our study included 50 patients 25 males and 25 females (age range 35-65year) and 26 females (age range 40-55 year) with chronic HCV infection admitted between 2013 and 2015 in El Hussein University Hospital (out and in patients of Tropical Department) disease duration 1.5-23y.

Fifty healthy individuals (25 males and 25 females) age, sex and socioeconomic condition were recruited for the study. Criteria for admission included a positive test for anti-HCV antibody for at least 6 months and HCV-RNA detectable in the serum. Diagnosis of chronic HCV liver disease was based on clinical, laboratory, and imaging evidence. The severity of the disease for patients with cirrhosis was assessed by calculating the Child-Pugh's score. According to Child-Pugh's score. they found 27 have child C, 16 have child B 11 child A.

Exclusion criteria:

1-Chronic kidney disease. -Endocrine disorders (Parathyroid, Thyroid disorders, primary biliary cirrhosis, Rheumatologic diseases, Metastatic bone disease or other malignancies.-Patients received hormone replacement therapy or corticosteroids.

All patients was subjected to the following:

1-Full personal and family history Clinical examination. General examination of musculoskeletal system to exclude other rheumatic diseases.

2) Bone mineral density:

Measurement of bone mineral density (g/cm^2) (BMD) at lumbar spines, Lt forearm, and Lt Femur using dual energy absorptiometry machine (DEXA) bone densitometry was performed using a LEXXOS 901 LX-157 medical device. All the subjects were classified according to WHO classification of osteoporosis: osteoporosis present when the a T score is less than -2.5 and osteopenia when the Tscore is between -1 and-2.5 15. (Blake and Fogelman, 2007)[7&12] **[15&61]**. None of the patients reported a past history or symptoms of bone fracture at the time of enrollment. All scans were carried out on the same machine by the same operator and were analyzed with the same software**.**

3) Laboratory assessment:

1-Level of serum 25-Hydroxycholecalciferol ng/ml [25-(OH)D] by Chemoillumicience technique. (5 ml of venous blood was collected from all patients and controls by clean venipuncture using plastic disposable syringes. Blood was allowed to clot before centrifugation and serum was taken. Serum concentration of 25(OH)-D was tested. (Arya, 2012).[6].

NB. Reference Values the following values are based on scientific literature and can be used as a guideline; (deficient <10-insufficient-10-30, sufficient 10-100, intoxication>100/25 OH ng/mL.

2-complete blood count 3) Coagulation tests. 4)-Serum level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), 5)-Total and direct bilirubin, 6)-gamma-glutamyltransferase (GGT), 7)-alkaline phosphatase (ALP). 8)- R.F, ANA, Anti-Ds DNA to exclude other rheumatic diseases. 9)-Kidney function to exclude causes of vitamin D deficiency.

Statistical analysis:

Statistical analysis was carried out using SigmaPlot® software (version 12.5, Systat Software, Inc., San Jose, CA 95110, United States). Quantitative data were expressed as descriptive values (mean, median, quartile, etc.), and represented in a box plot, and categorical data were presented in the form of frequencies. Non-parametric Chi square (χ^2) was used to detect if groups and independent variables (i.e. Osteoporosis and vitamin D scores)are associated at significant level of $p = 0.05$.

Data were summarized by median and range for continuous variables and frequency counts for categorical variables. Differences between groups

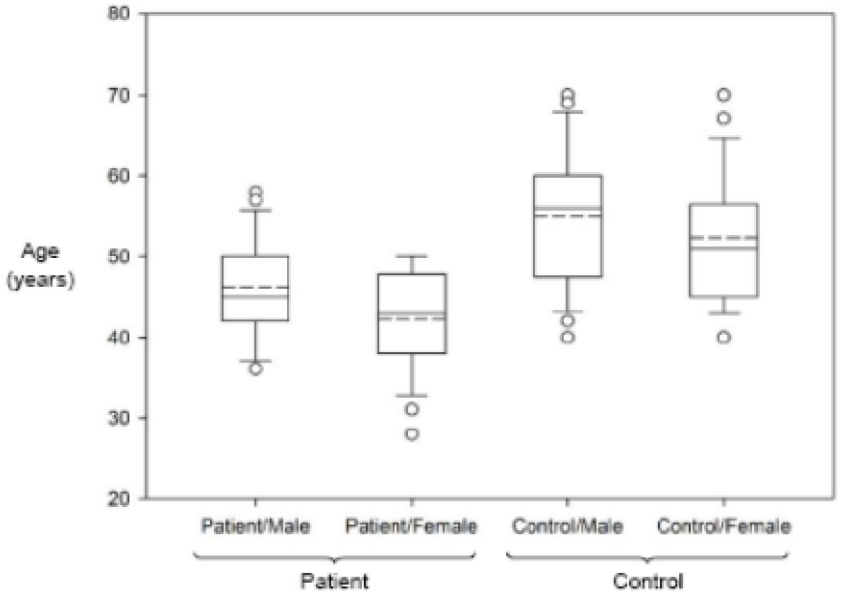
were assessed using Fisher's exact test for binary variables, the Mann-Whitney *U* test and the Kruskal-Wallis test for continuous variables. A *P*-value of <0.05 was considered statistically significant.

3. Results

Our study included 50 patients 24males (age range 35-65year) and 26 females (age range 40-55 year age) with chronic HCV infection and 50 control subjects. Disease duration 1.5-23y. Criteria for admission included a positive test for anti-HCV antibody for at least 6 months and HCV-RNA detectable in the serum. Diagnosis of chronic HCV liver disease was based on clinical, laboratory, and imaging evidence. Patients were classified according to Child-Pugh's score.

The results are showed in the following Tables and Figures.

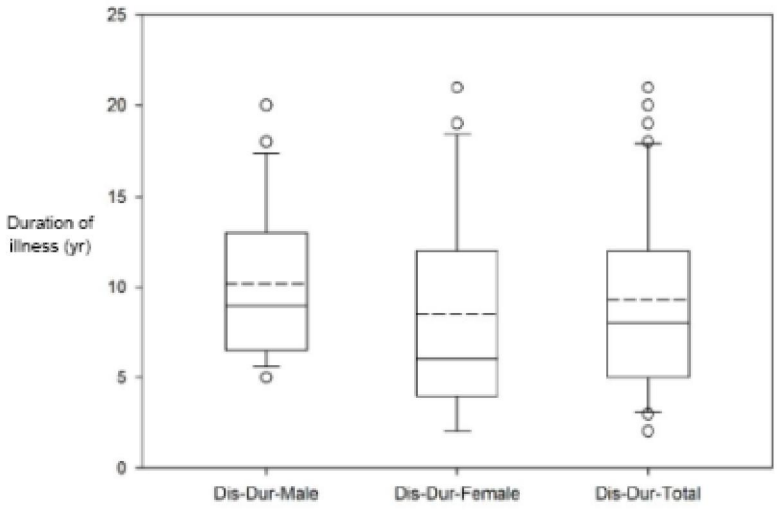
Table-1 & Fig.1: Descriptive and demo graphic data of studied groups' age (*n*=50/group) represented by a box plot constructed in Sigma Plot® 12.5 software.



Symbol	Denotation	Descriptive values of age			
		Patient		Control	
		Male	Female	Male	Female
▬	Mean	46.120	42.228	55.080	52.280
▬	Median	45.00	43.00	56.00	51.00
□	25th% - 75th% quartile	42.00 – 50.00	38.00–47.85	47.50–60.00	45.00–56.50
I	10th% - 90th% percentiles	37.00– 55.80	32.80–50.00	43.20– 67.80	43.00–64.60
o	Non-outliers (min. to max.)	36.00–58.00	28.00–50.00	40.00– 70.00	40.00– 70.00
	Standard Deviation (SD)	± 6.016	±6.413	± 8.326	±7.966
	Standard Error (SE)	±1.203	± 1.283	± 1.665	± 1.593
	Size (*n*)	25	25	25	25

Comment: The age of both male and females roughly symmetric around the median of each group of patient (45 and 43 years, respectively) and control (56 and 51 years, respectively) with a maximum and minimum range of 36 to 58 years for patients and 40 to 70 years for control.

Table-2, fig.2: Descriptive and demographic data of patients' disease duration within male (*n*=25), female (*n*=25) and in total.



Symbol	Denotation	Descriptive values stages of liver disease		
		A	B	C
---	Mean	5.842	8.889	12.50
———	Median	6.00	9.00	12.00
☐	25th% - 75th% quartile	4.00– 8.00	6.00–11.00	8.00 – 17.00
I	10th% - 90th% percentiles	2.00– 12.00	5.00–14.00	5.00– 19.00
o	Non-outliers (min. to max.)	2.00–13.00	5.00–14.00	3.00–21.00
	Standard Deviation (SD)	± 3.060	±2.892	± 5.226
	Standard Error (SE)	±0.702	± 0.964	±1.114
	Size (*n*)	19	9	22

Comment: The disease duration in males had a median of (9) with a minimum to maximum range of 5 to 20 years. While in females, median was (6) with a maximum to minimum range of 2 to 21 years.

Table-3: Descriptive and demographic data of different stages of liver disease, A (*n*=19), B (*n*=9) and C (*n*=22) at different disease durations.

Symbol	Denotation	Descriptive values stages of liver disease		
		A	B	C
---	Mean	5.842	8.889	12.50
———	Median	6.00	9.00	12.00
☐	25th% - 75th% quartile	4.00– 8.00	6.00–11.00	8.00 – 17.00
I	10th% - 90th% percentiles	2.00– 12.00	5.00–14.00	5.00– 19.00
o	Non-outliers (min. to max.)	2.00–13.00	5.00–14.00	3.00–21.00
	Standard Deviation (SD)	± 3.060	±2.892	± 5.226
	Standard Error (SE)	±0.702	± 0.964	±1.114
	Size (*n*)	19	9	22

Comment: A demoghragh showing the relation between the disease duration and CLD classification according to Child's classification. Group (A) with a median of 6 years, with a minimum to maximum of 2 to 13. Group (B) with median of 9, with minimum to maximum range of 5 to 14. Group (C) with a median of 12 years, with a maximum to minimum range of 3 to 21.

Tab- 4: Comparison between patients with osteoporosis and without osteoporosis

Clinical Data	with low BMD (n=28)		with normal BMD (n=22)		Controls
	Female	male	Female	male	
BMI (kg/m^2)	26.0 ± 2.5	27.9± 1.8	23.90 ± 28	27.0 ± 2	30.1± 1.8
Serum Ca (mg/dL)	8.8 ± 0.5	8.8 ± 0.7	8.9 ± 0.7	9.1 ± 0.3	9. ± 0.3
25-OH vit. D (ng/mL)	23.8 ± 8.9	28.7 ± 6.7	38.8 ± 5.9	36.7 ± 5.4	48± 5.4
Bone Alkaline Phosphatase (IU/L)	102± 20(IU/L	99± 54(IU/L	92± 45(IU/L	90± 11(IU/L	65± 5.4
AST(IU/L)	79± 66	87± 45	71 ± 32	69± 52	29.8± 32
S bilirubin (mg/dL	1.1 ± 0.78	1.2 ± 0.25	0.9 ± 0.67	1 ± 0.12	0.7± 32
INR	1.6 ± 0.05	1.2 ± 0.05	1.3 ± 0.05	0.9± 0.05	0.66± 32

Numbers are reported as mean ± SD. $P \leq 0.05$ (Significant)

Normal values ranges for: alanine aminotransferase, 0–42 IU/L; S. bilirubin, < 1.2 mg/dL; serum calcium, 8.62–10.20 mg/dL; bone alkaline phosphatase, 38–126 IU/L; serum 25-OH vitamin D, 30–40 ng/mL; International normalized ratio (INR).

Table -5: BMD in CPS classes and site of measurement (Lumbosacral Spine, Femoral Neck, lt forearm)

CPS	n	LS		Lt FN		Lt Forearm	
		BMD (g/cm^2)	**T score**	**BMD (g/cm^2)**	**T score**	**BMD (g/cm^2)**	**T score**
A	19 (38%)	0.94 ± 0.08	−0.55 ± 0.08	0.72 ± 0.09	−0.52 ± 0.03	0.72 ± 0.08	−0.52 ± 0.06
B	9 (19%)	0.89 ± 0.09	−0.97 ± 0.07	0.62 ± 0.08	−0.86 ± 0.06	0.62 ± 0.09	−0.87 ± 0.09
C	22 (44%)	0.84 ± 0.09	−1.59 ± 0.18	0.55 ± 0.03	−1.49 ± 0.13	0.65 ± 0.03	−1.48 ± 0.21
p		<0.009	< 0.012	< 0.0011	< 0.008	< 0.009	< 0.008

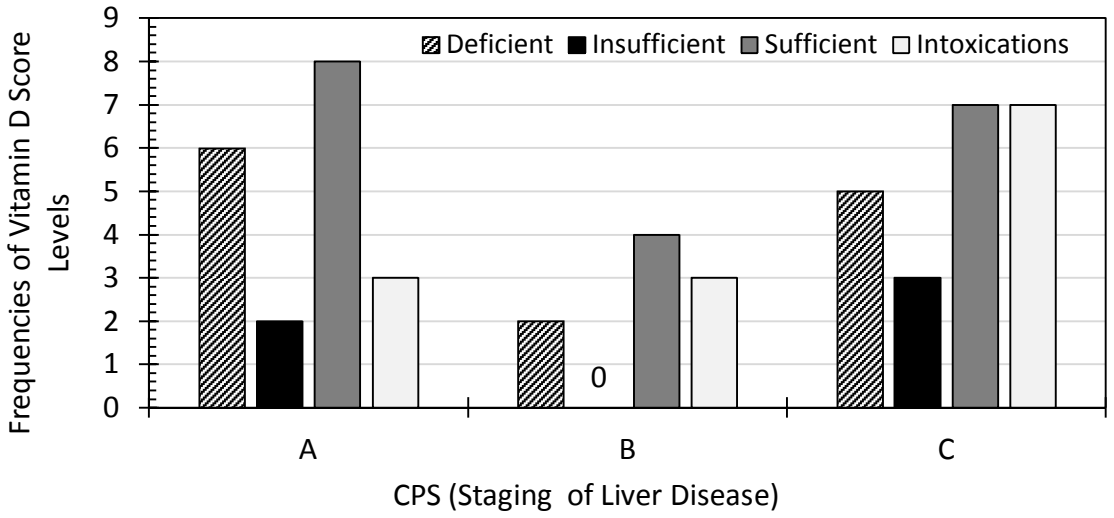
BMD=bone mineral density CPS Chronic liver stage. LS=lumbar spine FN=femoral neck the mean BMD values ($P < 0.001$), Z score values ($P < 0.001$) and T score values ($P < 0.001$) of the lumbar spine were significantly lower than those of the control group.

Table-6: Frequencies of osteoporosis and osteopenia scores within CPS (staging of liver disease).

Group	A(%)	B(%)	C(%)
N.	19(38%)	9(18%)	22(44%)
normal	14(73.7%)	5(55.%%)	3(13.7%
osteopenia	3(18.8%)	2(22.2%)	9(41%)
Osteoporosis	2(10.5%)	2(22.2%)	10(45.5%)

Comment: A graph showing the incidence of osteoporosis in relation to CLD staging (according to Child's classification). Patients under A class (n=19, 38%) showed 14 normal, 3 osteopenic and 2 osteoporotic. While those under (B) class (n=9, 18%) showed 5 normal, 2 osteopenic and 2 osteoporotic. Finally, patients under (C) class (n=22, 44%) showed 3 normal, 9 osteopenic and 10 osteoporotic.

Table-7, Fig.3: Frequencies of vitamin D score levels within CPS (staging of liver disease).



Groups	CPS (staging of liver disease)		
	A n=19	B n=9	C n=22
Deficient	6(31.8%)	2(22.2%)	5(22.7%)
Insufficient	2(10.5%)	0(0%)	3(13.6%)
Sufficient	8(42%)	4(44%)	7(31.8%)
Intoxications	3(15.7%)	3(33.3%)	7(31.8%)

Comment: A graph showing the frequency of vitamin D level of affection in patients with different CLD staging (according to CHILD'S classification). Patients under class (A) showed 6 patients with vi.t D deficiency, 2 with insufficiency, 8 were sufficient and 3 had intoxication levels. While patients under class (B) showed 2 patients with vit. D deficiency, No patients with insufficiency, 4 had sufficient levels of vit. D, and 3 showed intoxic levels. Patients under class (C) showed 5 patients with deficiency, 3 with insufficiency,7 with sufficient levels and 7 with intoxic levels.

Table-8: Frequencies of patients' gender within different range of osteoporosis scores.

Groups	Patients' Gender		Patients' Gender	
	Male =25	Female n=25	Male	Female
Normal	11(44%)	11(44%)		
Osteopenia	7(28%)	7(28%)		
Osteoporosis	7(28%)	7(28%)		

Comment: Graph showing the incidence of osteoporosis in relation to gender. Results showed that's the incidence of both osteopenia and osteoporosis are nearly equal in CL patients.

Table- 9, Fig.6: Frequencies of patients' gender within different vitamin D score levels.

Groups	Patients' Gender	
	Male(n%)	Female(n%)
Deficient	6(24%)	7(28%)
Insufficient	0 (0%)	5(20%)
Sufficient	11(44%)	8(32%)
Intoxications	8 (32%)	5(20%)

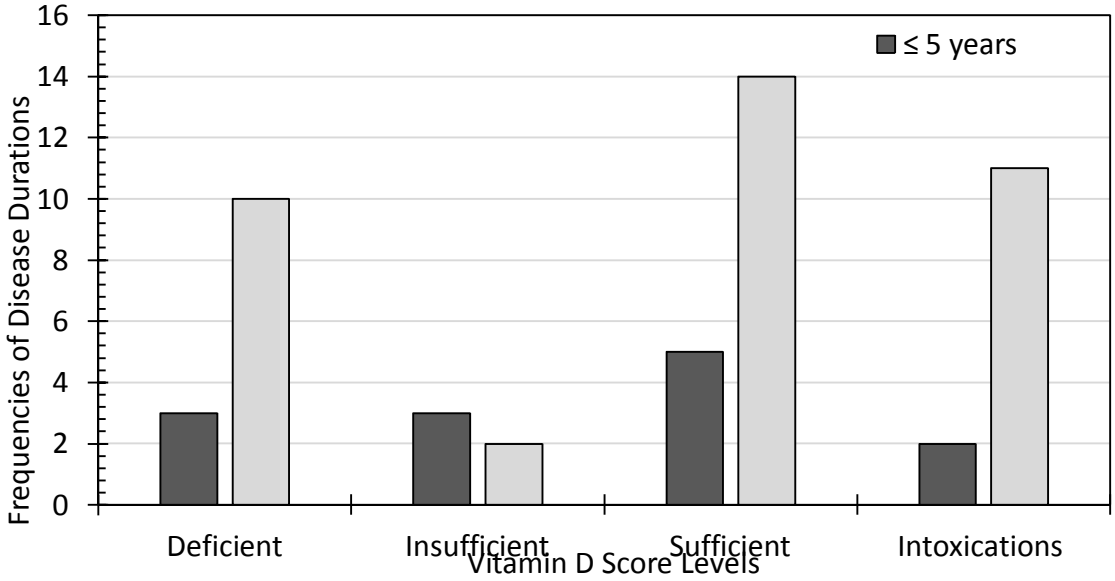
Comment: Graphic showing the incidence of vit. D level scoring in relation to patient's gender. Regarding vit. D. deficiency, 6 patients were males while 7 were females. In vit. D insufficiency, no males and 5 females. With vit. D sufficiency, 11 were males, and 8 were females. And with intoxication, 8 were males while 5 were females.

Table- 10: Frequencies of disease durations within different range of osteoporosis scores.

Groups	Disease Duration (Years)	
	≤ 5 n=13	> 5 n=37
Normal	9(69.2%)	13(35.1%)
Osteopenia	4(30.3%)	10(27%)
Osteoporosis	0(0.0%)	14(37.8%)

Comment: Demography showing the relationship between CLD duration and DEXA findings. In patients with disease duration less than or equal to 5 years, 9 patients had normal DEXA results, 4 showed osteopenia, and non had osteoporosis. For patients with disease duration more than 5 years, 13 patient had normal DEXA results, 10 had osteopenia, while 14 has osteoporosis.

Table-11, Fig.4: Frequencies of disease durations within different vitamin D score levels.



Groups	Disease Duration (Years)	
	≤ 5(n=13 & %)	> 5(n=37&%)
Deficient	3(6%)	10(20%)
Insufficient	3(6%)	2(4%)
Sufficient	5(10%)	14(28%)
Intoxications	2(4%)	11(22%)

Comment: A graphic showing the relationship between disease duration and vit D level scoring. In patients with disease duration less that, or equal to 5 years, (no=13 out of 50) 3 showed deficient vit. D levels, 3 showed insufficient vit D levels, 5 had sufficient levels and 2 with intoxic levels. While in patients with disease duration more than 5 years (no=37 out of 50), 10 patients showed vit. D deficiency, 2 showed vit. D insufficiency, 14 sufficient levels, and 11 with intoxic levels.

4. Discussion

Hepatitis C virus (HCV) infection affects approximately 184 million individuals worldwide; in some countries, up to 75% are unaware of their status due to the latent nature of disease progression Hanafiah et al., [16,]. Complications of chronic HCV infection include osteoporosis, with prevalence ranging from 14 to 28% Lin et al., [4], and increased fracture rates (Lo Re V 3rd et al., 2012) [14]. HCV infection mediates systemic immune activation and can lead to chronic liver dysfunction, both of which have notable effects on bone health. (Pacifici, 2010) [13].

Our Results showed that, the age of both male and females roughly symmetric around the median of each group of patient(45 and 43 years, respectively) and control (56 and 51 years, respectively) with a maximum and minimum range of 36 to 58 years for patients and 40 to 70 years for control Our results are in consistent with the literature proposing that HCV seroprevalence peaks after age 55 (Vasselle, et al., 2004)[15] Hanafiah et al., 2013[16] (Li et al.,

2013)[23] The mean age in the HCV exposure cohort was 54.1±15.3 years. our results revealed that most patients the mean age was 44 years, the females were in the child-bearing period. But Joseph et al[17] admitted that decrease in bone density will be after the age of 50 in studying both males and females. Our results are agreed with Lai et al, 2015 [28] 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. This in agreement with Lin, et al., 2012 [4] who stated T scores at the femoral neck and lumbar spine were used as the primary outcome variables to assess the association between degree of liver disease and BMD. The study cohort was 41 % male with a mean age of 53.6 years. The mean BMD, Z score, and T score values of lumbar spine in chronic hepatitis C (CHC) patients were significantly lower than those in healthy controls ($p < 0.001$). The rate of osteoporosis for CHC patients aged 45-54 years was significantly higher than that of the control group ($p = 0.011$). Patients with more advanced liver fibrosis had significantly lower BMD.

Rena and (David 2017) [24] reported approximately 75 to 85%, of persons infected with HCV will develop chronic infection. Factors associated with spontaneous clearance of HCV include younger age at infection, female sex, race other than African American, IL-28B CC genotype, and symptomatic acute infection. Our results Showed the relation between the disease duration and CLD classification according to Child's classification. Group (A) with a median of 6 years, with a minimum to maximum of 2 to 13. Group (B) with median of 9, with minimum to maximum range of 5 to 14. Group (C) with a median of 12 years, with a maximum to minimum range of 3 to 21. It is estimated that approximately 20 to 30% of those infected with HCV will develop cirrhosis during the 20 to 30 yearperiod after becoming chronically infected.(Rena and David 2017) [24].

Osteoporosis was defined as a BMD *T*-score of −2.5 or less or *Z*-score of −2.0 or less by DXA at either the spine or the hip [12]. Lucaci et al[18] and Schiefke et al[19] 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; Lin et al 2012 [4] and his collegues reported that, the mean BMD, Z score, and T score values of lumbar spine in chronic hepatitis C (CHC) patients were significantly lower than those in healthy controls ($p < 0.001$). The rate of osteoporosis for CHC patients aged 45-54 years was significantly higher than that of the control group ($p = 0.011$). Bone alkaline phosphatase and C-terminal cross-linking telopeptide of type I collagen levels were also significantly higher in CHC patients with reduced BMD. Patients with more advanced liver fibrosis had significantly lower BMD. this coincides with our results, the incidence of osteoporosis in relation to CLD staging (according to Child's classification). Patients under (A) class showed 14 normal, 3 osteopenic and 2 osteoporotic. While those under (B) class showed 5 normal, 2 osteopenic and 2 osteoporotic. Finally, patients under (C) class showed 3 normal, 9 osteopenic and 10 osteoporotic There was a significantly reduced BMD in non-cirrhotic patients with chronic hepatitis C infection.

HCV-infected subjects usually have vitamin D deficiencies, which are related to liver disease severity. However, there is inconsistency in the published data, possibly due to the heterogeneity in the study designs, such as characteristics of patients (HCV infection characteristics of HCV infection (genotype), and characteristics of vitamin D assessment (seasonality, cutoff values, and methodology). (Stokes, et al, 2013)[20] (Rahman, et al., 2013) [21]. The role of vitamin D in CHC patients is controversialand the mechanisms by which these associations occur are not well established.

On the other hand since the liver is responsible for 25(OH)D production, patients with liver injury may have low levels of.

25(OH)D due to low vitamin D absorption and impaired 25(OH)D synthesis.(Monica, et al., 2014)[22].

Vitamin D deficiency and insufficiency are common in cirrhotic populations with hepatitis C (HCV). The frequency of vitamin D level of affection in patients with different CLD staging (according to CHILD'S classification). Pateints underclass (A) showed 6 patients with vit D diffiency, 2 with insufficiency, 8 were sufficient and 3 had intoxication levels. While patients under class (B) showed 2 patients with vit D difficiency, No patients with insufficiency, 4 had sufficient levels of vit D, and 3 showed intoxic levels. Patients under class (C) showed 5 patients with difficinency, 3 with insufficiency, 7 with sufficient levels and 7 with intocant levels.

These results are inconsistence with Monica et al., 2014[22]. They reported, n early 70% of all patients had suboptimal 25(OH)D levels (below 20 or 30 ng/mL, and almost 50% of the HCV-infected patients had deficient 25(OH)D levels (less than 10 or 20 ng/mL).

The incidence of osteoporosis in relation to gender. Results showed that's the incidence of both osteopenia and osteoporosis are nearly equal in CLD patients.

Regarding the age, the study shows a clear influence of age on bone changes. It must not be forgotten that for older women menopause has an influence on BMD, which we did not consider in our study because the 2 groups (patients and controls) were age homogeneous.

Regarding vit D deficiency, 6 patients were males while 7 were females. In vit D insufficiency, no males and 5 females. With vit D suffiency, 11 were males, and 8 were females. And with intoxication, 8 were males while 5 were females.

Furthermore, Luchi et al., [18] reviewed 60 HCV patients, 34 of them had osteopenia, and 7 had osteoporosis. Schiefke et al[19] 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. These results are in accordance with our results**.** In patients with disease duration less than or equal to 5 years, 9 patients had normal DEXA results, 4 showed osteopenia, and non had osteoporosis. For patients with disease duration more than 5 years, 13 patient had normal DEXA results, 10 had osteopenia, while 14 has osteoporosis.

In patients with disease duration less that, or equal to 5 years, 3 showed deficient vit. D levels, 3 showed insufficient vit D levels, 5 had sufficient levels and 2 with intoxic levels. While in patients with disease duration more than 5 years, 10 patients showed vit D deficiency, 2 showed vit D insufficiency, 14 sufficient levels, and 11 with intoxic levels.

BMD and *T*-scores were lower in patients with chronic hepatitis C than in controls, In addition, we found a statistically significant difference between patients and controls ($P = 0.029$). Vit. D3 were lower in patients than in controls but no correlation was found between the two groups (P=0.47-0.56). our results are in agreement with, Lin et al[4] reported higher rates of osteopenia and osteoporosis in chronic HCV (Child-Pugh≤5) compared to the control group. Our results showed no significant differences of BMI between patients and controls but (Ecaterina-Constantaet al.,)[29] found a significant correlation between BMI BMD between patients and controles.

Conclusions

1-Chronic hepatitis C virus infection did increase the risk of development of metabolic bone disease in this cohort. Indeed, greater reduction of bone mass density occurs in advanced liver fibrosis. The bone loss in earlier stages of chronic hepatitis C infection is likely to result from increased bone reduction rather than decreased bone formation..

2-Our results suggest that, chronic viral C infection represents a risk factor for osteoporosis especially for patients with advanced liver disease, diagnosed for more than 5 years, and with a high viral Infections.

3-The relation between vitamin D deficiency and the degree of liver function, degree of fibrosis and infectious complications could support its use as a prognostic index and a diagnostic tool..

4-Our study showed an association between advanced stage of liver fibrosis, the decreased BMD level, and Vit D deficiency in chronic HCV patients.

References

1. S., Norris S., de Knegt R.J, Sanchez Avila J.F., Sonderup M, et. al.:J Viral Hepat. 2015 Jan;22 Suppl 1:6-25. doi: 10.1111/jvh.12350. Historical.
2. Mueller S., Millonig G., Seitz H.K.: Alcoholic liver disease and hepatitis C: a frequently underestimated combination. World J Gastroenterol 2009; 15: 3462-71.
3. Guichelaar M., Kendall R, Malinchoc M. and Hay J. E.: Bone mineral density before and after OLT: longterm follow-up and predictive factors. Liver Transpl. Sep 2006; 12(9):1390-402.
4. Lin J., Hsieh T., Wu C., Chen P., Chueh T., Chang W, et al.: Association between chronic hepatitis C virus infection and bone mineral density. Calcif Tissue Int. Dec 2012; 91(6):423-9.
5. Wariaghli G., Mounach A., Achemlal L., Benbaghdadi I., Aouragh A., Bezza A., El Maghraoui A.: Osteoporosis in chronic liver disease: a case-control study. Rheumatol Int 2010; 30(7): 893-9.
6. Arya, S. C.; Agarwal, N. (2012). "Pathology Consultation on Vitamin D Testing: Clinical Indications for 25(OH) Vitamin D Measurement". American Journal of Clinical Pathology. 137 (5): 832.
7. Blake G. and Fogelman I.: The role of DXA bone density scans in the diagnosis and treatment of osteoporosis. Postgrad Med J 2007; 83(982):509-17.
8. Metts J, Carmichael L, Kokor W, Scharffenberg R FP Essent. 2014 Dec; 427():25-31.
9. Backstedt D, Pedersen M., Choi M, and Seetharam A.: 25-Vitamin D levels in chronic hepatitis C infection: association with cirrhosis and sustained virologic response 2013;20(7):486-493. OR IGINAL AR T ICL E Annals of Gastroenterology (2017) 30, 1-5.
10. Averhoff FM, Glass N, Holtzman D Global burden of hepatitis C: considerations for

healthcare providers in the United States. Clin Infect Dis. 2012 Jul; 55 Suppl 10: S10-5.
11. E M.M, Samy S. Eldahdouh S.S. and Mohamed A.A.: The impact and effect of liver insufficiency of HCV infection on patients with chronic obstructive pulmonary diseases Egyptian Journal of Chest Diseases and Tuberculosis Volume 63, Issue 1, January 2014, Pages 81–85.
12. National Osteoporosis Foundation. Clinician's guide to prevention and treatment of osteoporosis. Washington, DC: National Osteoporosis Foundation; 2013.
13. Pacifici R.: Review T cells: critical bone regulators in health and disease. Bone. 2010 Sep; 47(3):461-71.
14. Lo Re V 3rd, Volk J, Newcomb CW, Yang YX, Freeman CP, Hennessy S, Kostman JR, Tebas P, Leonard MB, Localio AR: Risk of hip fracture associated with hepatitis C virus infection and hepatitis C/human immunodeficiency virus coinfection. Hepatology. 2012 Nov; 56(5):1688-98.
15. Vasselle C, Masini S, Bianchi F, et al. Evidence for association between hepatitis C virus seropositivity and coronary artery disease. Heart 2004; 90:565–566.
16. Hanafiah KM, Groeger J, Flaxman AD, et al. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology 2013; 57:1333–1342.
17. Joseph Melton, Elizabeth A. Chrischilles, Cyrus Cooper, Ann W. Lane and B. Lawrence Riggs. How Many Women Have Osteoporosis? Journal of Bone and Mineral Research 2005; 20: 886-892.
18. Luchi S, Fiorini I, Meini M, Scasso A. Alterazioni del metabolismo osseo in pazienti con epatite cronica da virius C. Infez Med. 2005; 1: 23-27.
19. 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: 1843-1847.
20. Stokes CS, Volmer DA, Gr€unhage F, Lammert F. Vitamin D in chronic liver disease. Liver Int 2013;33:338-352.
21. Rahman AH, Branch AD. Vitamin D for your patients with chronic hepatitis C? J Hepatol 2013;58:184-189.
22. Monica G-A, Daniel P-T, Marıa A. J-S, Amanda F-R, Marıa G-F, and Salvador R.: Relationship of Vitamin D Status With Advanced Liver Fibrosis and Response to Hepatitis C Virus Therapy: A Meta-analysis Hepatology, volume 60 Issues 29 Sep 2014.
23. Li D, Long Y, Wang T, et al. Epidemiology of hepatitis C infection in highly endemic HBV areas in China. Plos ONE 2013; 8: e54815.
24. Rena k F. and David S.H. Evolution and staging and monitoring of Chronic Hepatitis C march 15,2017 Hepatitis on line.
25. Abu-Mouch S., Fireman Z., Jarchvsky J., et al.: Vit D supplementation improves sustained virologic response in chronic Hepatitis C (genotype 1)-nave patients World Gastroentrology 2011;24:5184-90.
26. V., Del Campo J.A., Ranchal I., Lampe E., Romero-Gomez M.: Association between vitamin D and hepatitis C virus infection: a meta-analysis. World J Gastroenterol. 2013 Sep 21;19(35):5917-24. doi: 10.3748/wjg.v19.i35.5917.
27. Terrier B., Carrat F., Geri G., et al.: Low 25-OH vit D serum levels correlate with severe fibrosis in HIV-HCV coinfected patients with chronic hepatitits J.Hepatol. 2011;55:756-61..
28. L, Shoback DM, Zipperstein J, Lizaola B, Tseng S,Terrault NA: Bone Mineral Density, Bone Turnover, and Systemic Inflammation in Non-cirrhotics with Chronic Hepatitis C. Dig Dis Sci. 2015 Jun;60(6):1813-9.
29. Ecaterina-Constanta., Cristina-Emilia C-T., Mihai L.,Ramona S. P.1,3, Adrian O. A., Daniela A. I., Ioana A.B. The Effect of Chronic Viral Hepatitis B and C on Bone Mineral Density. Revista Romana DE Boli InfecºIoase – V. XVIII, Nr. 4, 2015.
30. Hansen AB, Omland LH, Krarup H, et al. Fracture risk in hepatitis C virus infected persons: results from the DANVIR cohort study. J Hepatol 2014; 61:15–21.

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