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ORIGINAL ARTICLE

Evaluation of Bone Mineral Density in Non-Alcoholic Fatty Liver Disease using Dual Energy X-Ray Absorptiometry

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Abstract

Background: Nonalcoholic fatty liver disease (NAFLD) has become a health epidemic involving not only the developed but also the developing world. It is a metabolic syndrome associated with variety of manifestations including hypovitaminosis D through a variety of metabolic mechanisms. Hypovitaminosis D has a direct effect on the bone mineral density (BMD) of patients. Latter can be easily estimated objectively through dual x-ray absorptiometry (DXA) of the lumbar spine and right hip. Though majority of the studies including few Indian studies have shown inverse relationship between NAFLD & BMD, yet few studies fail to show any such correlation. Hence, we conducted a study to find the association between NAFLD and BMD.

Materials and Methods: Seventy cases of NAFLD along with 35 healthy subjects were included in our hospital-based, case-control study which was conducted over a period of 18months following approval from institutional ethics committee and after obtaining an informed consent. Patient were included after using a strict inclusion and exclusion criteria while healthy subjects were included after ruling out NAFLD. Ultrasonography and laboratory investigations were used for diagnosis of NAFLD while DXA was used to assess the BMD in lumbar spine & right hip region in both cases and controls.

Results: The study revealed significant association between NAFLD and reduced BMD, though no linear association was noted between fatty liver and BMD. Statistically significant difference was observed between BMD of patients with grade II & III fatty liver and grade I & III fatty liver. Patients with NAFLD had statistically significant low vitamin D levels compared to healthy controls. However, difference in serum calcium levels were not statistically significant in the case & control groups. Patients with NAFLD have statistically significant elevated BMI, total cholesterol and liver enzymes compared to healthy controls.

Conclusion: NAFLD shows a linear relationship with BMD inferring that with increase in the severity of NAFLD, there is significant reduction in the BMD. Though this reduction is associated with hypovitaminosis D yet serum calcium levels remain largely unaltered leading to osteoporosis and increase risk of bone fractures. Hence, this knowledge may help the clinicians in better management of the patients with NALFD, thereby reducing the morbidities related to hypovitaminosis D especially those related to osteopenia and osteoporosis.

Keywords: Bone mineral density, non-alcoholic fatty liver disease, dual energy x-ray absorptiometry

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Introduction

Fatty liver disease refers to fat accumulation within the hepatocytes [1]. It is commonly associated with alcoholic and nonalcoholic fatty liver disease (NAFLD) [2]. NAFLD is diagnosed following exclusion of alcoholic fatty liver, autoimmune hepatitis, primary biliary cirrhosis and

Wilson's disease [3]. NAFLD is a metabolic disorder commonly associated with metabolic syndrome, central obesity, type-II diabetes mellitus, hypertension, insulin resistance and dyslipidemias [4]. It ranges from simple steatosis (fatty liver without cellular injury) to nonalcoholic steatohepatitis (NASH) which leads to scarring, fibrosis and cirrhosis [5] which may be a precursor of hepatocellular carcinoma [6].

Prevalence of NAFLD is around 25.24% worldwide [6,7] and 9-32% [3,6] in India with higher prevalence among overweight/obese and prediabetic/diabetics[8–12].

Although the pathophysiology of NAFLD is still unclear, the so called "Multiple-hit hypothesis" describes it as a complex, two-step liver injury [13]. The first hit is characterized by insulin resistance leading to hepatic triglyceride accumulation leading to steatosis; followed by the "second hit" of adipocytokine-induced liver injury, oxidative and endoplasmic reticulum stresses, mitochondrial dysfunction, and hepatic apoptosis, promoting simple steatosis to steatohepatitis [14–16]. Insulin resistance (IR) plays a central role in both first and second hits [17]. Insulin resistance and hyperinsulinemia increase the excretion of triglycerides by the liver, resulting in elevated serum levels of triglycerides [18,19].

NAFLD and Osteoporosis

Recent epidemiological studies show that NAFLD is linked with osteoporosis not only in adults and postmenopausal women but also in pediatric population due to the following postulated associations:

(a) *Hypovitaminosis D*: Serum levels of 25-hydroxyvitamin D is lower in patients with NAFLD/NASH as compared to healthy controls [20–22].

(b) Growth Hormone-Insulin Growth Factor-1 (GH/IGF-1) Axis: Both GH and IGF-1 are of great importance in hepatic physiology, mainly due to the finding that IGF-1 is the main mediator of GH activity in subjects with NAFLD. Several studies have shown that low levels of IGF-1 are associated with a greater risk of vertebrae and hip fractures because bone tissue is the second richest source of IGF-1, and IGF-1 directly promotes osteoblast differentiation and growth [23–25].

(c) Circulating Molecules and Chronic Inflammatory Process: Most of the systemic inflammation pathways and mediators involved are common for both NASH and inflammatory osteoporosis. Among many molecules, tumor necrosis factor-alpha (TNF-á) is the key factor in both mediating and inducing hepatocyte death as well as a classical inducer of inflammatory osteoporosis.

Evaluating NAFLD By Ultrasonography

Ultrasonography is the most widely used non-invasive technique to diagnose NAFLD with has a sensitivity of 60-94% and specificity of 88-95% [26,27]. Ultrasonographic evidence of fatty liver with laboratory evidence of increased enzymes is used for diagnosis of NAFLD after excluding hepatitis B and C, autoimmune hepatitis, hemochromatosis and cystic fibrosis.

Evaluating Bone Mineral Density by Dual Energy X-Ray Absorptiometry (DXA)

DXA scan measures the bone mineral density in grams and bone area in square centimeter, then calculates "areal" BMD in gm/cm^2 by dividing bone mineral content and bone area. T-score and Z-score are calculated and used for the diagnosis of osteoporosis. The Third National Health and Nutrition Examination Survey (NHANES III) reference database is utilized for T and Z score derivation [28].

Recently, several studies have demonstrated that hypovitaminosis D could play an important role in the development of insulin resistance and non-alcoholic fatty liver disease [29–32]. Many studies have shown positive association between NAFLD and osteoporosis [33–35]. Cui R et al found that NAFLD has a detrimental effect on bone mineral density in males and females [33]. Sun Y et al in their meta-analysis indicated that obese children with NAFLD had lower BMD and Z-score than the control group [36]. Conversely a meta-analysis of five cross-sectional studies showed no significant difference in BMD between patients with and without NAFLD [37].

Hence, we aimed to evaluate bone mineral density in patients with Nonalcoholic fatty liver disease (NAFLD) using Dual energy X-ray absorptiometry (DXA).

Materials and Methods

This hospital-based, case control study was performed on 70 cases and 35 controls in the Department of Radiodiagnosis of our institution over a period of one and a half years following approval from ethics review committee and after obtaining informed consent.

Inclusion criteria for NAFLD

- Ultrasonographic evidence of hepatic steatosis
- Mild to moderately elevated liver enzymes
- Abnormal lipid profile in absence of history of alcohol intake (even if less than once a week), use of any hepatotoxic drug, Seropositivity for hepatitis B or C, Congenital liver diseases (Wilson's disease - low ceruloplasmin level, hemochromatosis-

transferrin saturation >45).

Exclusion criteria for NAFLD

- Past history of long-standing immobilization
- Current smokers
- Postmenopausal old women were excluded from the study.
- History of drug intake like vitamin D supplements, interferons, corticosteroids, etc. which may affect bone turnover.

Selection of Controls

Thirty-five, healthy, age and sex matched volunteers in whom NAFLD was ruled out based on clinical, laboratory and/or sonographic finding were taken as controls.

As a measure of obesity, body mass index was calculated by using formula weight in kilograms and height in m^2. Fasting blood total cholesterol, Liver function test, (serum alanine aminotransferase (ALT), aspartate aminotransferase (ASP), alkaline phosphatase (ALP)), serum calcium levels and vitamin D were measured in all enrolled cases & controls. Serology for hepatitis B & C, Antinuclear antibody, Transferrin saturation and α-1 Antitrypsin deficiency were also rule out.

Ultrasonography of liver

Ultrasonography (USG) of liver was performed by an experienced radiologist blinded to patient's clinical information using curvilinear 3-5MHz probe **(GE LOGIC S8**) to assess & grade fatty changes in the liver as below (Image 1):

- **GRADE I or mild steatosis:** Diffusely increased liver echogenicity (higher than adjacent right kidney) with normal periportal and diaphragmatic visualization.
- **GRADE II or moderate steatosis:** Diffusely increased liver echogenicity obscuring periportal echogenicity but with preserved diaphragmatic visualization.
- **GRADE III or severe steatosis:** Diffusely increased liver echogenicity obscuring periportal echogenicity and diaphragmatic visualization.

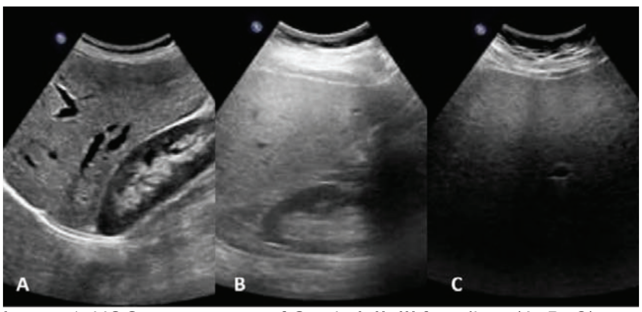

Image 1: USG appearance of Grade I, II, III fatty liver (A, B, C)

DXA for Bone Mineral Density (BMD)

BMD was measured by using Dual energy x-ray absorptiometry *(HOLOGIC DISCOVERY Wi USA)*. The BMD of the lumbar spine (L) and right hip (RH) were evaluated, and their means served as mean BMD in gm/cm^2.

T-score, the value used for the diagnosis of osteoporosis was calculated by:

$$\text{T-score} = \frac{\text{Measured BMD} - \text{Young adult mean BMD}}{\text{Young adult population SD}}$$

Z-score was used to compare the patients BMD to a population of peers, and calculated by:

$$\text{Z-score} = \frac{\text{Measured BMD} - \text{Age matched mean BMD}}{\text{Age matched population SD}}$$

The Third National Health and Nutrition Examination Survey (NHANES III) reference database was utilized for evaluation of T and Z scores.

The World Health Organization definitions of osteoporosis and osteopenia were used to interpret spine and hip on Dual-energy x ray absorptiometry (DXA) scan. (Table 1)

Table 1: WHO definitions of osteopenia and osteoporosis on DXA

Terminology	T-score Definition
Normal	T ≥ -1.0
Osteopenia	-2.5 ≤ T ≤ -1.0
Osteoporosis	T ≤ -2.5

The recorded data was analyzed by appropriate statistical tests. A p-value of less than 0.05 was considered statistically significant. All P-values were two tailed.

Results

In our study, the age of cases ranged from 21 to 50 years (mean age-40.07) and age of controls ranged from 22 to 51 years (mean age-41.3). There was no statistically significant difference in age of cases and controls (p = 0.62).

In our study, 24 (34.3%) cases were males, and 46 (65.7%) cases were females. Among controls, 11(31.4%) were males and 24 (68.6%) females. There was no statistically significant difference in gender of cases and controls (p = 0.769).

In our study, mean AST, ALP, ALT of cases was 35.20, 108.33 and 66.49IU and mean AST, ALP, ALT of controls was 28.17, 78.29 and 42.54IU respectively. There was statistically significant difference of liver enzymes in cases and controls (p=0.045), (p=0.002), (p=0.037) respectively.

In our study, mean cholesterol level in cases was 253.77 mg/dl and mean cholesterol level in controls was 138.09 mg/dl. There was statistically significant difference in mean cholesterol levels of cases and controls (p<0.001).

In our study, mean vitamin D levels in cases was 25.32 ng/dl and mean vitamin D levels of controls was 37.68 ng/dl. There was statistically significant difference in vitamin D levels of cases and controls (p<0.001).

In our study, mean calcium level in cases was 9.39 mg/dl and mean calcium level in controls was 9.72 mg/dl, which was not statistically significant (p=0.068).

In our study, the BMI of cases ranged from 20.56-37.74 with most cases being overweight/obese. BMI of controls ranged from 18.98-27.34. There was statistically significant difference in BMI of cases and controls (p-value <0.001).

In our study, 5 patients of grade I (7.1%), 48 patients of grade II (68.6%) and 17 patients Grade III (24.3%) were included.

In our study, mean LS T-score & Z- score of cases was -2.00 & -0.83 respectively while mean LS T-score & Z-score of controls was -0.44 & 1.30 respectively with statistically significant difference (p<0.001).

In our study. mean T-score & Z-score of RT hip of cases was 1.09 & 0.32 respectively while mean T-score & Z-score of RT hip of controls was 1.25 & 1.09 respectively with statistically significant difference (p<0.001).

In our study, mean LS BMD for cases was 0.815 and LS BMD for controls was 0.996 with a statistically significantly difference (p<0.001).

In present study mean RT hip BMD for cases was 0.788 and mean BMD for controls was 0.974. There was statistically significant difference in lumbar BMD of cases and controls (p<0.001).

In present study, mean average BMD of cases was 0.801 and for controls was 0.985. There was statistically significantly difference in mean lumbar BMD of cases and controls (p<0.001). Thus, patients with NAFLD had statistically significant lower BMD compared to healthy controls.

In present study, mean BMD of grade I fatty liver was 0.885, for grade II fatty liver was 0.845, and for grade III fatty liver was 0.711. There was no statistically significant difference in mean bone mineral density of cases and controls who had grade I and grade II fatty liver, however significant statistical difference was observed between mean bone mineral densities of patients of grade II and grade III and grade I and grade III fatty liver. (p-value = 0.259), (p-value=0.014) and (p- value=0.017) respectively (Table 2).

Table 3 shows the distribution of the various study parameters among cases and controls with statistical p-value while Table 4 shows the distribution of the various study parameters in cases and controls based on sex.

Discussion

Non-alcoholic fatty liver disease (NAFLD) is a common cause of liver disease worldwide, affecting approximately 20-35% of adults in general population [6]. It is a hepatic manifestation of metabolic syndrome which comprises type II diabetes mellitus, hypertension, insulin resistance, central obesity and dyslipidemias.

Our study had a age range of 21-50years with mean of 40years in cases while the age range among controls is 22-51 years with mean 41.3 years with no statistical difference. The study conducted by Cui R et al however had a mean age of 60.23years and 59.42 years in case and control groups respectively [33]. The reason for this difference is exclusion of postmenopausal old women.

In our study, 24 (34.3%) were males & 46 (65.7%) were females in case group while 11 (31.4%) males and 24 (68.6%) females among controls with no statistical difference. However, NAFLD was commoner in females. Purnak T et al in their study has findings consistent with our study [35].

In our study, weight of patients with NAFLD was significantly higher than control group. Cui R et al in their study had findings comparable with our study [33].

In our study, mean height has no significant relation with

Table 2: Showing BMD according to Grade of Fatty Liver in Cases

Grade of fatty liver	N	Mean BMD	SD	Comparison	Difference	p-value
Grade I	5	0.885	0.047	Grade I vs Grade II	0.040	0.259
Grade II	48	0.845	0.169	Grade II vs Grade III	0.134	0.014*
Grade III	17	0.711	0.146	Grade I vs Grade III	0.174	0.017*

Table 3: Comparison of various parameters between cases and controls

	Cases		Controls		p-value
	Mean	SD	Mean	SD	
Age (yrs)	40.7	6.52	41.3	7.52	0.623
Weight (Kg)	75.1	8.97	62.8	6.93	<0.001*
Height (Cm)	156.7	2.07	157.2	1.69	0.219
BMI (kg/m^2)	30.7	3.671	23.4	2.03	<0.001*
Lumbar spine BMD (g/cm^2)	0.815	0.168	0.996	0.131	<0.001*
Right hip BMD (g/cm^2)	0.788	0.176	0.974	0.170	<0.001*
Mean BMD (g/cm^2)	0.801	0.155	0.985	0.123	<0.001*
Vitamin D (ng/dl)	25.32	21.53	37.68	29.54	0.016*
Total Cholesterol (mg/dl)	253.77	100.71	138.09	61.94	<0.001*
Calcium (mg/dl)	9.39	0.861	9.72	0.896	0.068
AST (IU/L)	35.20	16.41	28.17	17.58	0.045*
ALP (IU/L)	108.33	46.00	78.29	46.37	0.002*
ALT (IU/L)	66.49	59.21	42.54	44.47	0.037*

Table 4: Comparison of various parameters between cases and controls based on gender

Variables	Male			Female		
	Case	Control		Case	Control	
	Mean±SD	Mean±SD	P value	Mean±SD	Mean±SD	P value
Age (Yrs)	39.88±8.00	43.91±5.58	0.141	41.09±5.66	40.21±8.10	0.598
Weight (Kg)	77.46±8.05	66.27±6.84	<0.001*	73.78±9.24	61.25±6.51	<0.001*
Height (Cm)	167.8±2.38	169.1±1.86	0.124	148.5±2.18	149.2±1.78	0.181
BMI (kg/m^2)	31.17±3.63	24.36±1.69	<0.001*	30.48±3.80	23.04±1.94	<0.001*
Lumbar spine BMD (g/cm^2)	0.828±0.19	0.999±0.13	0.013*	0.809±0.15	0.995±0.13	<0.001*
Right hip BMD (g/cm^2)	0.818±0.16	1.020±0.21	0.005*	0.772±0.18	0.952±0.15	<0.001*
Mean BMD (g/cm^2)	0.822±0.17	1.009±0.13	0.004*	0.789±0.15	0.974±0.12	<0.001*
Vitamin D (ng/dl)	25.34±21.34	28.30±25.86	0.724	26.05±27.68	41.73±34.54	0.043*
Total Cholesterol (mg/dl)	247.1±95.35	153.1±84.17	0.008*	257.2±104.3	131.2±49.34	<0.001*
Calcium (mg/dl)	9.53±0.96 9	.42±0.82	0.744	9.32±0.81	9.86±0.91	0.012*
AST (IU/L)	32.42±14.69	32.00±18.11	0.943	33.61±17.38	26.42±17.43	0.105
ALP (IU/L	111.9±52.01	84.55±50.01	0.153	106.5±43.03	75.42±45.42	0.006*
ALT (IU/L	68.58±62.07	46.82±53.36	0.323	65.39±58.34	38.58±40.90	0.048*

occurrence of NAFLD similar to that seen by Purnak T et al [35].

In our study as well as that of Umehara T, statistically significant difference was noted in mean BMI of case and control group [38].

In our study, statistically significant difference of liver enzymes in cases and controls was noted with p-values for AST, ALP and ALT to be 0.045, 0.002, and 0.037 respectively. These findings are consistent with Pardee PE et al who also showed increase in liver enzymes in NAFLD patients as compared to controls [34]. Umehara T et al found p-value of <0.001 for evaluation of raised liver enzymes in case and control group [38]. Similarly, Xia MF et al concluded that elevation of the hepatotoxic biomarker ALT may indicate high risk for osteoporosis in patients with NAFLD [39].

In our study, mean cholesterol level difference among cases and controls was statistically significant similar to a study by Wang Y et al [40]. However, they found no significant association in women group. In contrast, we excluded postmenopausal women who generally have raised serum cholesterol levels. Our study levels are also coherent with Cui R et al [33] and Dennison EM et al that found inverse relation between serum cholesterol and BMD [41].

In our study, mean vitamin D levels difference was statistically significant among cases similar to that seen by Eshraghain A et al [42] who found significantly lower levels of vitamin D in case group. In multiple studies including Rhee EJ et al [43], it has been found that supplemental vitamin D can reduce risk of fatty liver, steatohepatitis and osteoporosis. Wang X et al in their systemic review of 29 studies showed reduced vitamin D levels in NAFLD patients which are in concordance with our results. [44] Barchetta I et al showed patients with NAFLD had reduced serum D levels compared with subjects without NAFLD similar to our results reinforcing strong association between hypovitaminosis D and NAFLD [45].

Our study revealed no significant difference in mean calcium level among case and control group similar to Purnak T et al [35]. This is because calcium is kept with a narrow range by hormones acting on bone calcium to maintain serum calcium levels leading to osteoporosis without significant changes in serum calcium levels.

In our study, the difference in mean LS/RT Hip T-score & LS/RT Hip Z-score as well as LS/RT Hip BMD was statistically significant, meaning thereby patients with NAFLD have reduced bone mineral density compared to healthy controls. Our findings were consistent with Xia MF et al [39]. Similarly, Wang Y et al concluded that osteoporotic fractures occur more commonly in patients with NAFLD [40]. Moon SS et al in their retrospective study suggested that postmenopausal women with NAFLD may have higher risk of osteoporosis than those without NAFLD [46]. Postmenopausal women were however excluded in our study. Yang HJ et al in their retrospective study concluded that NAFLD was negatively associated with the right-hip BMD [47].

In our study, grade I, II & III fatty livers were found in about 5 (7.1%), 48 (68.6%) and 17 (24.3%) cases respectively. Our study showed mean BMD (sum of mean BMD of lumbar spine & right hip divided by 2) in grade I, II & III is 0.885, 0.845 and 0.711 respectively with no statistically significant difference in LS BMD of cases and controls who had grade I vs II fatty liver. However significant statistical difference was observed between BMD of case and control in grade II vs III and grade I vs III fatty liver changes indicating that increase in grade of fatty liver is associated with reduced bone mineral density. Sethia R et al found inverse relationship between BMD and severity of NAFLD as well as grade of fatty liver [48].

Our study findings are similar to the study conducted by Chen HJ et al who found that the risk of new-onset osteoporosis was higher in patients with NAFLD than in the comparison cohort [49]. In addition, the difference of this incidence remained significant in the follow-up durations of one year and more than 10 years. Patients with NAFLD bear 1.35 times higher risk of subsequent osteoporosis than those without NAFLD (95% confidence interval=1.20-1.53). Mantovani A et al showed significant difference in whole body and lumbar BMD Z-scores between children/adolescents with and without NAFLD [50]. Similarly, Sun Y et al found that obese children with NAFLD are more susceptible to osteoporosis than children with only obesity [36]. Umehara T et al found significant correlation between NAFLD and reduced bone marrow density [38]. Xia MF et al found that liver fat content is inversely related to BMD, which is in concordance with our study [39]. Chen HJ et al found increased risk of osteoporosis in patients with NAFLD [49]. Similarly Mantovani A. et al found significant association between NAFLD and reduced BMD in children [50]. Khukhlina OS et al found inverse relationship between BMD with NAFLD [51]. However, a minuscule of studies including Bhat SP et al and Chang EJ et al have shown results which are discordant to that of the above studies with no obvious correlation between NAFLD and BMD [52,53].

Limitations of Study

- Factors affecting BMD including physical activity & exposure to sunlight were not taken into consideration
- USG liver instead of liver biopsy was used to diagnose NAFLD.

Conclusion

Nonalcoholic fatty liver disease is significantly associated with reduced bone mineral density. Also, patient with NAFLD have increased BMI, blood cholesterol, liver enzymes and reduced serum Vitamin D levels.

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Ethics:	There is no ethical violation as it is based on voluntary anonymous interviews
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