Sarcopenia and Bone Mineral Density in MAFLD Patients

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Abstract:

Background: MAFLD affects about quarter of the world's adult population, decreased bone mineral density and lean body mass share some common underlying mechanisms with MAFLD and it's important to study the relation between these three conditions to design more effective management strategies for patients. AIM: This study aims to evaluate skeletal muscle mass and function together with bone mineral density in patients with MAFLD. **Patients and Methods:** This cross-sectional study was performed on 50 patients diagnosed with MAFLD, Bioelectrical Impedance Analysis (BIA) was done to assess appendicular skeletal muscle mass (ASM) and muscle strength was assessed by chair stand test (CS-30), bone mineral density (BMD) was assessed by Quantitative Ultrasound (QUS). **Results:** The mean age ±SD was 51.98± 10.96; there were 28 % male, 72 % female. There was statistically significant correlation between S3 steatosis and HBA1c & INR. Serum albumin was significantly lower in patients with F4 fibrosis. HBA1c and ALP were significantly higher in patients with Sarcopenia. Risk of osteopenia and osteoporosis increase in MAFLD patients with advancing age. Receiver operating characteristic (ROC) analysis was performed to determine the value of BMD using QUS (T score) in prediction of sarcopenia. BMD at a cut off value 88.4 had 80% sensitivity& 60% specificity, with AUC was 0.636, and was non-significant (P = 0.143). **Conclusion:** MAFLD is associated with decreased skeletal muscle mass & function and BMD, so management strategies for patients with MAFLD should include screening and management for sarcopenia and low BMD.

Introduction

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Metabolic associated fatty liver disease (MAFLD), formerly named non-alcoholic fatty liver disease (NAFLD), is the most common chronic liver disease and affects appropriately 30 % of the general population globally[1].

Widespread adoption of the name and definition of MAFLD allows greater standardization across the spectrum of disease and realizes cogent, coherent and logical framework to understand, diagnose and treat this common disease [2].

MAFLD is associated with an increased risk of cirrhosis, liver cancer, type 2 diabetes (T2DM), and atherosclerosis[3].

sarcopenia is characterised by progressive and systemic reduction in skeletal muscle mass, strength or function, with an increased risk of disability, hospitalisation and mortality[4].

Sarcopenia and NAFLD share similar underlying mechanisms and risk factors, including insulin resistance, chronic inflammation, vitamin D deficiency, and physical inactivity [5].

Osteoporosis is characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility [6]. Given the high prevalence of both NAFLD and osteoporosis, it is not surprising that these two conditions may coexist especially in the aging population, the two diseases share common risk factors, including but not limited to aging, sedentary lifestyle, and sex hormone deficiencies, suggesting that they may be linked beyond a simple coincidence [7].

However, despite the high prevalence and clinical significance of osteoporosis and sarcopenia in patients with liver disease, attention and management strategies for these musculoskeletal disorders are frequently overlooked in clinical practice for patients with liver disease [8].

Patients and Methods
Study design:
This is a cross-sectional study that was carried on consecutive 50 patients diagnosed with MAFLD at Hepatology and gastroenterology outpatient clinics at Mahalla Hepatology Teaching Hospital, the study was initiated in January 2023 to August 2023. All procedures performed in studies involving human participants were in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. The study protocol was approved from the ethical committee of scientific research of Benha faculty of medicine. (Study no. Ms.22.12.2022)

Study population:
Patients were chosen according to the following inclusion and exclusion criteria:

Inclusion Criteria:
Metabolic associated fatty liver disease (MAFLD) criteria include adult patients aged $\geq$ 18 years with hepatic steatosis (steatosis detected by either imaging abdominal ultrasound, blood biomarkers and scores) with the presence one of the three criteria, overweight or obesity, type 2 diabetes mellitus or evidence of metabolic abnormalities such as an increased waist circumference and an abnormal lipid or glycaemic profile[1].

Exclusion criteria:
We excluded Patients with:
1. Age less than 18 years.
2. Active malignancy.
3. Pregnancy.
4. Known cases of osteoporosis.
5. Patients with end stage organ failure e.g. congestive heart failure and renal failure.
6. Other causes of sarcopenia such as cancer.
7. Patients receiving drugs affecting muscle mass and bone mineral density e.g. steroids.

Methods
Clinical assessment:
All patients were subjected to medical history taking including age, sex, current drugs, smoking and alcohol consumption. Body mass index (BMI) was calculated as body weight (kg) divided by body height (m^2). Waist circumference was measured at a level midway between the lower rib margin and iliac crest with the tape all around the body. Blood pressure was measured on both arms in the sitting position after resting.

Laboratory assessment:
Laboratory investigations included ALT, AST, ALP, serum albumin, serum bilirubin and INR. Blood was sampled for assays of fasting Triglycerides, cholesterol, HDL, LDL, glucose after fasting for 8 hours over night, HbA1c, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and liver function tests. Liver fibrosis was also assessed using clinical noninvasive scores of fibrosis:

1. The AST to platelet ratio index (APRI) was calculated as :
- AST (upper limit of normal)/platelet count ($\times 10^9$/l)$\times$100. Fibrosis index for liver fibrosis.

2. Fibrosis index for liver fibrosis (FIB-4) will also be calculated as :
- Age $\times$ AST (IU/l)/platelet count ($\times 10^9$/l)$\times$ALT (IU/l).

3. The NAFLD fibrosis score (NFS) was calculated as :
- 1.675+0.037$\times$ (age, year)+0.094$\times$(BMI, kg/m2)+1.13$\times$impaired fasting glucose/diabetes (yes=1, no=0)+0.99$\times$AST/ALT ratio–0.013$\times$(platelet count, $\times$109/L)–0.66$\times$(albumin, g/dL) [9].

4.The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) formula :
- HOMA-IR = (fasting insulin (μIU/ml) X fast- fasting glucose (mmol/l))/22.5 10 .

Radiological assessment:
Abdominal ultrasonography

The patients were divided into three groups according to the grade of steatosis:
- **Grade 1**: Mild steatosis.
- **Grade 2**: Moderate steatosis.
- **Grade 3**: Severe steatosis [11].

FibroScan examination
During the hepatology clinic visit, liver stiffness measurement (LSM) and controlled attenuation parameter (CAP) obtained using FibroScan 502. All patients were fasting for at least 8 hours before the procedure, the LSM score was represented by the median of 10 measurements and was considered reliable only if at least 10 successful acquisitions are obtained and the interquartile range (IQR) to median ratio of the 10 acquisitions are ≤0.3, LSM of patients was graded to F0, F1, F2, F3, and F4 disease b 5.9 ± 1. 8, 7.3 ± 2.8, 8.7 ± 3.4, 11.2 ± 3.8, and 21.2 ± 14.7 kPa, respectively. The CAP score was represented by the median value. CAP measurements were considered reliable and included in the final analysis if 10 successful acquisitions were obtained. Hepatic steatosis was graded by CAP using the M probe according to published cutoffs (S1=222–232; S2=233–289; S3 ≥290 dB/m) [12] .

Sarcopenia assessment :
Sarcopenia was diagnosed as loss of muscle strength or impaired physical performance, namely "low muscle function" and low muscle mass [13].

low muscle mass was assessed by **Bioimpedance Analysis (BIA)** as Skeletal muscle mass (SMM) can be taken directly from the report of a BIA device and used as the appendicular skeletal muscle mass as defined by European Working Group on Sarcopenia in Older People or Asian Working (EWGSOP) Group for Sarcopenia [14].

Low muscle strength was assessed by chair stand test as the **Chair Stand test (CS-30)** was found to be a beneficial diagnostic tool for assessing the risk of sarcopenia [15].

Bone Mineral Density assessment:

Bone Density Meter Quantitative Ultrasound (QUS) bone densitometry for assessment of bone mineral density. Calcaneal QUS was proved to be a method of osteoporosis pre-screening that could be widely promoted in the general population, especially the middle aged and elderly, due to its portability, low cost and ease of use [16].

Statistical analysis

Data were fed to the computer and analysed using IBM SPSS software package version 20.0 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp). Qualitative data were described using numbers and percentages. The Shapiro-Wilk test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Significance of the obtained results was judged at the 5% level. The used tests were Chi-square test, Student t-test, Mann Whitney test, Analysis of variance [ANOVA] tests and The Kruskal-Wallis test.

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Results
Baseline characteristics:
This study was done on 50 adult patients with mean age 51.98± 10.96 years ranged from 30 to 73 years. The results showed that as regards to fibrosis grading there were 60% F0- F1, 12% F2, 10% F3, 18% F4, (the commonest was F0-F1). Regarding to Steatosis grading there were 8%S1, 30% S2, 62% S3, (the commonest was S3). Regarding to US grading there were 8% G1, 30% G2, and 62% G3. Regarding HbA1c and INR were statistically significant in grade S3 steatosis. (p≤0.05).(Table 1).

Table (1): Relation between the degree of steatosis and baseline laboratory parameters.

	Degree of steatosis									Test value	P-value
	S1			S2			S3				
	Mean	±	SD	Mean	±	SD	Mean	±	±SD		
Triglycerides (mg/dl)	133.40	±	55.08	108.30	±	33.08	100.34	±	42.74	KW=2.976	0.226
HDL (mg/dl)	47.25	±	9.74	45.40	±	4.56	49.90	±	16.55	KW=0.871	0.647
LDL (mg/dl)	121.8	±	34.4	127.2	±	34.4	114.5	±	48.9	F=0.429	0.654
Cholesterol (mg/dl)	211.22	±	45.96	190.06	±	47.40	182.10	±	49.11	F=0.689	0.507
HOMA-IR	4.09	±	5.84	3.76	±	4.09	4.63	±	4.86	KW=1.281	0.527
CRP (mg/L)	Negative			Negative			Negative				-
ALT (IU/L)	29.0	±	15.3	21.9	±	10.5	20.3	±	8.0	KW=2.654	0.261
AST (IU/L)	27.59(IU/L)	±	14.71	22.48	±	10.13	21.67	±	7.99	KW=0.426	0.808
ALP (IU/L)	78.04	±	26.98	92.43	±	34.60	101.59	±	41.32	KW=2.632	0.268
FBS (md/dl)	93	±	13	104	±	30	117	±	37	KW=1.319	0.517
HBA1C	5.58	±	0.78	5.40	±	0.83	6.30	±	1.11	KW=4.205	**0.021**
S. albumin	4.06	±	.28	3.88	±	1.10	4.37	±	.32	KW=4.517	0.104
S. bilirubin	.63	±	.15	.74	±	.82	.46	±	.20	KW=4.914	0.086
INR	1.03	±	.02	1.04	±	.04	1.07	±	.04	KW=6.400	**0.041**
APRI	.3066	±	.042	.9240	±	2.2399	.2776	±	.2051	KW=3.811	0.149
FIB4	1.4863	±	.4393	1.5111	±	1.2677	1.2271	±	.7625	KW=1.296	0.523
NFS	2.455	±	.939	1.860	±	1.507	1.968	±	1.645	KW=0.737	0.692

p≤0.05 is statistically significant, p≤0.01 is high statistically significant, SD: standard deviation, KW: Kruskal Wallis Test, F: One-Way ANOVA Test

While serum albumin was significantly lower in grade F4 (p=0.033), NFS was significantly higher in grade F4 (p=0.032). Appendicular skeletal muscle mass using bioelectrical impedance ranged from 17.3 to 39.9 with mean of 26.36± 5.3, muscle function using chair stand test in 30s ranged from 5 to 19 with mean of 9.64± 2.51, sarcopenia including both low muscle power & mass was reported in 5 (10%) of NAFLD patients (Table 2). Bone mineral density scan shows that 11 patients (22%) have osteopenia and only one patient (2%) had osteoporosis the rest of patients have normal BMD (Figure 1).

There was no significant relation between degree of fibrosis and ASM as well as BMD score (p>0.05) (Table 3). There was no significant relation between grades of steatosis and ASM as well as BMD T score (p>0.05) (Table 4). Receiver operating characteristic (ROC) analysis was performed to determine the value of BMD using QUS (T score) in prediction of sarcopenia. BMD at a threshold value 88.4 had 80% sensitivity& 60% specificity, with AUC was 0.636, and was non-significant (P = 0.143) (Table 5) and (Figure 2).

Table (2): Parameters of sarcopenia among the studied patients.

Parameters		Studied patients (N= 50)
Appendicular skeletal muscle mass(ASM) (kg)	**Mean± SD**	27.12± 6.38
	Range	17.4 – 53.2
Muscle function (chair stand test in 30 sec)	**Mean± SD**	9.56± 2.5
	Range	5.0 – 19.0

SD= standard deviation, n: number, %: percentage,

Table(3): Relation between the degree of fibrosis, ASM and BMD

		grades of fibrosis						Test value	P-value
		Mild fibrosis (F0-F1) N=30			**Advanced fibrosis (F2-F4) N=20**				
		Mean	**±**	**SD**	**Mean**	**±**	**SD**		
ASM		27.18	±	5.71	25.14	±	4.65	T=1.329	0.19
BMD score	**T**	94.13	±	16.48	89.195	±	17.05	T=1.023	0.31

p≤0.05 is statistically significant, p≤0.01 is high statistically significant, SD: standard deviation,T: Student T Test

**Table (4):** Relation between the grades of steatosis, ASM and BMD

		grades of steatosis						Test value	P-value
		Mild and moderate steatosis (S1 – S2) N=19			**severe steatosis (S3) N=31**				
		Mean	**±**	**SD**	**Mean**	**±**	**SD**		
ASM		26.01	±	6.196	26.58	±	4.87	T=0.362	0.719
BMD score	**T**	88.89	±	14.39	94.15	±	17.92	T=1.082	0.285

p≤0.05 is statistically significant, p≤0.01 is high statistically significant, SD: standard deviation: Student T Test

Table (5): Validity (AUC, sensitivity, specificity) for BMD (using QUS,T score in prediction of sarcopenia.

	Best cut off	Sensitivity	Specificity	PPV	NPV	AUC	P-value
BMD (using QUS,T score)	88.4	80%	60%	66.7%	75%	0.636	0.143

AUC: Area Under a Curve　　　　　　　　p value: Probability value
NPV: Negative predictive value　　　　　　PPV: Positive predictive value
*: Statistically significant at p ≤ 0.05
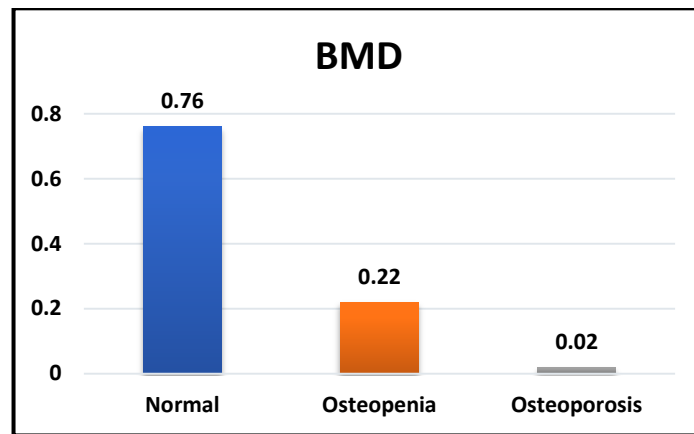
Figure (1): Distribution of studied patients regarding BMD.
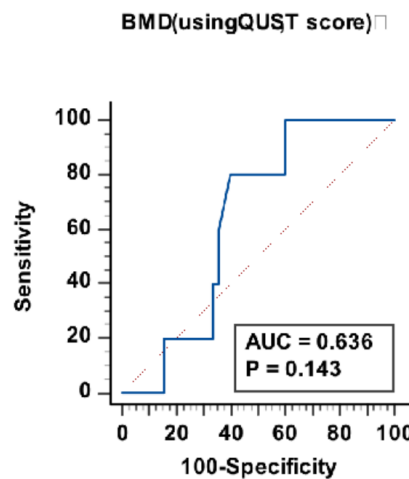
Figure (2): ROC curve of BMD using QUS (T score) in prediction of sarcopenia.

Discussion

Metabolic dysfunction associated fatty liver disease (MAFLD) is the most common liver disease affecting a quarter of the global population and is often associated with adverse health outcomes. The increasing prevalence of MAFLD occurs in parallel to that of metabolic syndrome, which in fact plays a major role in driving the perturbations of cardiometabolic homeostasis. However, the mechanisms underpinning the pathogenesis of MAFLD are incompletely understood [17]. Bone and the skeletal

muscle are fat free tissues which appeared to be independently associated with MAFLD in several cross-sectional studies, The deterioration of bone mineral density and lean body mass leading to osteoporosis and sarcopenia respectively, are age related processes [4]. The current study aimed to evaluate skeletal muscle mass and function together with bone mineral density in patients with MAFLD. Regarding the demographic data the results showed that the mean age ±SD being 51.98± 10.96, there were 28% male, 72% female, ranged from 30 to 73 years. The results showed that as regards to fibrosis grading there were 60% F0- F1, 12% F2, 10% F3, 18% F4, (the commonest was F0-F1), Regarding to steatosis grading there were 8%S1, 30% S2, 62% S3, (the commonest was S3). This comes in agreement with researchers [18] who found that steatosis grades (0-3) < 5% 0 5%-33% 1 34%-66% 2 > 66% 3 , and with others[19] who found that fibrosis stages were 43 % F1, 25% F0, 13 % F2, 10 % F3 and 9% F4. (The commonest was F1). The current study showed that HbA1c was significantly higher in grade S3 steatosis, this finding is in agreement with Seo, et al [20], INR was significantly higher in grade S3 steatosis, in contrast with [21] who found that NAFLD patients were more likely to have a lower INR than patients without NAFLD, this is explained with only a few studies have focused on the complication of NAFLD As they have reported, patients with NAFLD are often accompanied by a decline in liver function, and an increased activity of some circulating coagulation factors (FVIII, FIX, FXI and FXII) has been found in patients of NAFLD which may also affect INR[21].Meanwhile, there was no significant relation between degree of steatosis and S. triglycerides, LDL, HDL, HOMA-IR, Cholesterol, AST, ALT, FBS, ALP, S. albumin, S. bilirubin and other laboratory data (p>0.05), Our results consistent with other researchers [22] who found that there was no significant relation

between degree of steatosis and LDL, HDL, Cholesterol and albumin. In This study, serum albumin was significantly lower in grade F4 (p=0.033), while NFS was significantly higher in grade F4 (p=0.032) which is in consistent with other study[22]. Meanwhile, there was no significant relation between degree of fibrosis and other laboratory data (p>0.05). In agreement with our results, a study was done[23] where it was reported that there was no significant relation between degree of fibrosis and alanine aminotransferase and triglycerides. In the current study, appendicular skeletal muscle mass (ASM) using bioelectrical impedance ranged from 17.3 to 39.9 with mean of 26.36± 5.3 which is less than that reported in a study performed by a group of researchers [24] (30.7%) but more than that reported in a study performed by researchers[25] (22.3%), this is explained that degree of liver fibrosis differed significantly according to sarcopenia status in subjects with MAFLD, which strongly suggests that the amount of skeletal muscle mass needs to be assessed for long-term risk stratification in subjects with MAFLD [22]. The muscle function using chair stand test in 30s ranged from 5 to 19 with mean of 9.64± 2.51, low muscle power was reported in 18 (36%) of NAFLD patients. This finding is in agreement with the study that reported that patients with 5 times chair to stand test >15 sec (40%) had significantly higher liver stiffness measurement[26]. In the present study, sarcopenia including both low muscle power & mass was reported in 10% of NAFLD patients. This finding is consistent with others [27] who found that the prevalence of sarcopenia among the participants with MAFLD was 8·8 %. In the present study, there was significant relation between sarcopenia and gender as sarcopenia was more detected in females and there was no significant relation between sarcopenia and age, DM, hypertension (p>0.05) which is consistent with a study previously performed [28]. This study showed that there was significant

relation between sarcopenia and BMI & waist circumference. These results are consistent with others [24] who reported that there was significant difference between sarcopenia gender, BMI and waist circumference. This study showed that HbA1c was significantly higher in cases with sarcopenia (p =0.020), in agreement with a previous study [29] who reported that high HbA1c levels, pre-diabetes, diabetes and diabetes complications were associated with an increased risk of sarcopenia. ALP was significantly higher in cases with sarcopenia (p=0.006), as reported before [30]. Meanwhile, there was no significant relation between sarcopenia and other laboratory data (p>0.05). Our results are consistent with others[28] who reported that there was no significant relation between sarcopenia and triglycerides, ALT, AST, HDL, albumin and total bilirubin. In this study, The BMD T. score ranged from 53.5 to 125.7 with mean± sd of 92.15± 16.72, bone mineral density scan shows that osteopenia was found in 11 (22%) of NAFLD patients and one case (2%) had osteoporosis, which is less prevalent than a study performed by Hassan et al [31] who aimed to determine whether Upper Egyptian patients with MAFLD are at risk of developing osteoporosis. They found that among the NAFLD group of 50 patients, 19 patients had osteoporosis and 28 patients had osteopenia, this can be explained that patients with NAFLD tend to have a significant decrease in bone density, vitamin D, and serum calcium levels [31]. This study showed that age was statistically higher in osteopenia and osteoporosis (p=0.003) which is consistent with the researchers of a study[32]. Meanwhile, there was no significant relation between BMD findings and other demographic features including gender, smoking, DM, hypertension, BMI as well as waist circumference, this is similar to a study which was performed previously [31]. In addition, there was no significant relation between BMD findings and

laboratory data. Our results are consistent with the former study[32]. In this study, there was no significant relation between degree of steatosis with ASM as well as BMD. This is similar to a study performed by others [33] who found that there was no significant relationship between the degree of hepatic steatosis and BMD. In contrast with our results, the scientists [34] who found that ASM gradually decreased as the severity of hepatic steatosis increased, as the ASM increased, the percentages of subjects with mild and severe disease were gradually decreased. ASM was independently associated with the severity of hepatic steatosis by logistic regression analysis [34].

In addition to our results, there was no significant relation between degree of fibrosis with ASM as well as BMD. This is similar to a study performed previously [33] However, Guo, et al [34] who found that individuals with F2-F3 and F4 liver fibrosis groups had significantly lower ASM than individuals with F0-F1 stages, As the SMI increased, the percentages of subjects in F2 and F3-F4 stage were gradually decreased. ASM was independently associated with the severity of hepatic fibrosis by logistic regression analysis. Moreover, decreased ASM was an independent risk factor for NAFLD and fibrosis [34].

This study showed that there was no significant relation between presence of sarcopenia and BMD score, which is similar to a study performed before[32]. Receiver operating characteristic (ROC) analysis was performed to determine the value of BMD using QUS (T score) in prediction of sarcopenia. BMD at a threshold value 88.4 had 80% sensitivity & 60% specificity, with AUC was 0.636, and was non- significant (P = 0.143). Our results consistent with others [35] who aimed to identify screening test criteria and cut-off values for osteosarcopenia those are feasible for epidemiological study and screening purposes. They found that receiver

operating characteristic (ROC) analysis was performed to determine the value of BMD using QUS (T score) in prediction of sarcopenia. BMD had 87.5 sensitivity& 90.2% specificity.

Conclusion:

MAFLD is associated with decreased skeletal muscle mass & function and BMD, so management strategies for patients with MAFLD should include screening and management for sarcopenia and low BMD

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