

Serum Vitamin D Level in Lean and Obese Patients with Metabolic Associated Fatty Liver Disease: A comparative study

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Abstract

Background and aim: Metabolic dysfunction associated fatty liver disease (MAFLD) affects around one third of the world population. Within the MAFLD population, 19.2% are lean. Low serum vitamin D concentrations were reported to increase the risk of MAFLD. This study aimed to explore the association between serum vitamin D concentration and MAFLD. **Methods:** This cross-sectional study was conducted on 50 Egyptian patients with lean MAFLD (BMI<25 kg/m²) (G I) and another group (G II) including 50 consecutive overweight/obese patients with MAFLD (BMI ≥25 kg/m²). MAFLD patients were evaluated by thorough history taking, full clinical examination, laboratory investigations including serum level of 25 hydroxycholecalciferol by ELISA, abdominal ultrasonography and FibroScan[®] with controlled attenuation parameter (CAP). **Results:** Males were significantly predominant in the lean group (G I) (60%) while females were significantly predominant in G II (62%). Mean serum vit D level was not significantly higher in G I compared to GII (16.38 and 15.44 ng/ml, respectively). Vitamin D deficiency (level <20 ng/ml) was predominant in G II (70% vs 58.0% in GI) while insufficiency (level: 20-30 ng/ml) was more common in GI (34% vs 26%). Sufficient vitamin D (level >30 ng/ml) was only found in 8% of GI compared to 4% of GII. Serum vitamin D level showed a highly significant negative correlation with steatosis grades in both groups (r=0.87& 0.88 in GI &GII respectively, P-value <0.001 in both groups). **Conclusion:** MAFLD patients, whether lean or obese, show low serum vitamin D levels, which negatively correlate with steatosis grades.

Keywords: Metabolic Associated Fatty Liver Disease (MAFLD); Lean MAFLD; Obese MAFLD; Vit; Controlled Attenuation Parameter (CAP) and FibroScan.

Introduction

Metabolic associated fatty liver disease (MAFLD) has emerged as the most common cause of liver diseases globally⁽¹⁾ and it is predicted to become the leading cause of liver transplantation by 2030⁽²⁾.

The diagnosis of MAFLD is based on the detection of liver steatosis (liver histology, non-invasive biomarkers or imaging together with the presence of at least one of three criteria that includes overweight or obesity, type 2 diabetes mellitus (T2DM) or clinical evidence of metabolic dysfunction which was defined by the presence of at least two of the following metabolic risk abnormalities: 1) waist circumference $\geq 90/80$ cm in Asian men and women; 2) blood pressure $\geq 130/85$ mmHg or specific drug treatment; 3) plasma triglycerides ≥ 1.70 mmol/L or specific drug treatment; 4) plasma HDL-cholesterol < 1.0 mmol/L for men and < 1.3 mmol/L for women or specific drug treatment; 5) prediabetes (i.e., fasting glucose levels 5.6 to 6.9 mmol/L, or 2-h post-load glucose levels 7.8 to 11.0 mmol or HbA1c 5.7% to 6.4%); 6) plasma high-sensitivity C-reactive protein (hs-CRP) level > 2 mg/L; and 7) homeostasis model assessment (HOMA)-insulin resistance score ≥ 2.5 ⁽³⁾.

MAFLD is no longer a diagnosis of exclusion and is based on the presence of metabolic dysfunction, it is now possible to diagnose its coexistence with other liver diseases unlike Nonalcoholic Fatty Liver Disease (NAFLD) which was defined as the presence of $> 5\%$ of hepatic steatosis (HS), in the absence of competing liver disease etiologies, such as chronic viral hepatitis, use of medications that induce

steatosis such as amiodarone or tamoxifen, and other chronic liver diseases (CLD), such as autoimmune hepatitis, hemochromatosis, Wilson's disease (WD), or significant alcohol consumption⁽⁴⁾.

Recently the 3 acronyms, metabolic dysfunction-associated steatotic liver disease (MASLD), MetSLD, or metabolic steatotic liver disease (MSLD) can be used as the replacement term for NAFLD and metabolic dysfunction-associated steatohepatitis (MASH) as the replacement term for NASH⁽⁵⁾

Although overweight/obesity is closely associated with the development and progression of MAFLD, subtle weight gain that has not led to overweight is an important determinant of incident metabolic disease and MAFLD. Within the MAFLD population, 19.2% of people are lean and 40.8% are non-obese, without difference in the histological severity of disease between lean and obese patients^[6,7]. Up to one-third of patients with MAFLD and a normal BMI meet the criteria for metabolic syndrome. Therefore, the identification, diagnosis, and treatment of non-obese MAFLD is very important^[7].

The risk factors for non-obese MAFLD remain unclear. Some previous cross-sectional studies showed that vitamin D deficiency was associated with an increased risk of NAFLD, and vitamin D levels were negatively associated with the severity of NAFLD^(8,9).

Vitamin D has been associated with many diseases' pathogenesis including autoimmune disease, cardiovascular

disease, cancers, inflammatory processes, and liver diseases ^(10,11).

The pathogenesis of the association between MAFLD and low vitamin D levels is undetermined; however, protective anti-fibrotic and anti-inflammatory function of vitamin D on the hepatic stellate cells has been suggested ⁽¹²⁾. Vitamin D reduces free fatty acid-induced insulin resistance in peripheral tissues and in hepatocytes ⁽¹³⁾. Therefore, low vitamin D level may lead to intrahepatic lipid accumulation which is responsible for NAFLD pathogenesis ⁽¹⁴⁾.

The aim of the present study was to explore the association between serum vitamin D concentration and MAFLD comparing lean and obese patients.

Patients and methods

This cross-sectional study was carried out on 100 patients with MAFLD attending the outpatient's clinic of the Hepatology, Gastroenterology, and Infectious Diseases Department of Benha University Hospitals, Egypt, within the period from February 2022 to February 2023. They were subdivided into 2 groups; lean group (G I) comprised 50 consecutive lean - (BMI < 25 kg/m²) and obese group (G II) comprised 50 consecutive obese - (BMI > 30 kg/m²) MAFLD patients. An informed written consent was obtained from each participant before the study. The whole protocol was approved by the Ethical Committee of Benha Faculty of Medicine, Benha University }M.S24.4.2022}.

Inclusion criteria:

- Adult patients of both genders with MAFLD.

Exclusion criteria:

- Age less than 18 years.

- Pregnant females.
 - Advanced comorbid illnesses and malignancies.
 - Decompensated liver cirrhosis.
 - History of intake of steatogenic drugs (amiodarone, tamoxifen, corticosteroids, estrogens,....ect).
 - History of intake of vitamin D supplementation (within at least 6 months before the study)
 - Diseases affecting vitamin D metabolism such as (malabsorption, chronic kidney disease, pancreatic and hepatobiliary disease, ...ect).
- All the studied patients were subjected to the following:

1-Thorough history taking.
2-Complete general examination with stress on: •Blood pressure: Average resting blood pressure (BP) was obtained from 3 measurements made with a standard mercury sphygmomanometer at 3-minute intervals.

- BMI.
- Waist circumference.
- Abdominal examination with stress on: hepatomegaly and splenomegaly.

4-Anthropometric measurements

Each subject underwent a physical examination. Measurements of weight (to the nearest 0.1 kg) and height (to the nearest 0.1 cm) were used to calculate the BMI [= (kg/m²)]. Waist circumference (W) was measured on the midaxillary line between the lower border of the rib cage and the upper margin of the iliac crest (15).

5-Biochemical assessments

After overnight fasting, serum was collected. Biochemical markers, including

Total cholesterol (TC), Triglyceride (TG), High density lipoprotein (HDL), Low density lipoprotein (LDL), Fasting blood glucose (FBG), HbA1c, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Serum level of active form of Vitamin D was done using ELISA technique (NOVA, Beijing, China)

MAFLD Diagnosis

The diagnosis of MAFLD was based on the detection of liver steatosis by imaging (abdominal ultrasound and FibroScan with CAP (FibroScan expert, Echonsens, Paris, France) together with the presence of at least one of three criteria that includes overweight or obesity, type 2 diabetes mellitus (T2DM) or clinical evidence of metabolic dysfunction.

Metabolic dysregulation was defined by the presence of at least two of the following metabolic risk abnormalities: 1) waist circumference $\geq 90/80$ cm in Asian men and women; 2) blood pressure $\geq 130/85$ mmHg or specific drug treatment; 3) plasma triglycerides ≥ 1.70 mmol/L or specific drug treatment; 4) plasma HDL-cholesterol < 1.0 mmol/L for men and < 1.3 mmol/L for women or specific drug treatment; 5) prediabetes (i.e., fasting glucose levels 5.6 to 6.9 mmol/L, or 2-h post-load glucose levels 7.8 to 11.0 mmol or HbA1c 5.7% to 6.4%);

6- Serum vitamin level assessment by ELISA

(NOVA, Beijing, China) where *Sufficient (>30 ng/ml) *Insufficient (20-30ng/ml) *Deficient (<20 ng/ml).

7- Abdominal ultrasonography (LOGIQ, Korea)

8-FibroScan with CAP

(FibroScan expert, Echonsens, Paris, France).

F0 (0_5.4 kPa) F1 (5.5_6.9 kPa) F2 (7_8.9 kPa) F3 (9_ 11.4 kPa) F4 (11.5_75 kPa)

S0 (0_222 dB/m) S1 (223_ 259 dB/m) S2 (260_310 dB/m) S3 (311 _400 dB/m)

Statistical analysis:

These data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 26. Descriptive statistics were calculated for the data in the form of mean, standard deviation (\pm SD) and number and percentage. In the statistical comparison between the different groups, the significance of difference was tested using student's t-test to compare between mean of two groups of numerical (parametric) data. For continuous non-parametric data, Mann-Whitney U- test was used for inter-group analysis, data. Inter-group comparison of categorical data was performed by using chi square test (X^2 -value), Pearson correlation coefficient (r) test was used correlating different parameters. P value <0.05 was considered statistically significant.

Results

Table (1) Comparison between the studied groups regarding demographic data and routine laboratory findings

	Lean Group N0=50	(GI)	Obese group N0=50	(GII)	Test of sig.	p-value
Age (Ys) (mean \pm SD)	41.16	10.79	45.60	11.99	t=1.9	0.06
Gender						
Male	30	60.0%	19	38.0%	X ² =4.8	0.03*
Female	20	40.0%	31	62.0%		
Smoking (No. & %)	17	34.0%	12	24.0%	X ² =1.2	0.3
Hypertension (No. & %)	13	26.0%	19	38.0%	X ² =1.6	0.2
Haemoglobin (g/dl)	12	\pm 1.1	12	\pm 11.2	1.8	0.07
WBCs x (10 ³)/ mm ³	5.8	\pm 1.2	6.1	\pm 1.08	1.5	0.1
Platelet count x(10 ³)/ mm ³	274	\pm 36	264	\pm 35.9	1.3	0.2
ALT (U/L)	28	\pm 13	29	\pm 14.5	0.4	0.7
AST (U/L)	28	\pm 13.7	31	\pm 13.2	1.1	0.3
HbA1C (%)	5.7	\pm 0.68	5.8	\pm 1.2	1.2	0.2
FBG (mg/dl)	96	\pm 17.6	104	\pm 27	1.6	0.1
2HPP (mg/dl)	170	\pm 182	145	\pm 40	0.9	0.4
S. creatinine (mg/dl)	0.98	\pm 0.15	0.98	\pm 0.14	0.2	0.8
Cholesterol (mg/dl)	229	\pm 51.3	226	\pm 47.8	0.3	0.8
TG (mg/dl)	201	\pm 57.1	197	\pm 79.9	0.3	0.8
LDL (mg/dl)	127	\pm 28.8	131	\pm 29.3	0.7	0.5
HDL (mg/dl)	33	\pm 7.4	34	\pm 16.5	0.4	0.7
Vit D level (ng/ml)	16.38	\pm 8.42	15.44	\pm 7.21	1.8	0.07

Table (2): Comparison between mean 25 hydroxy vitamin D level in different fibrosis stages and steatosis grades in the studied groups.

	Group I N0=50	Group II N0=50	T	p-value
	Mean \pm SD	Mean \pm SD		
Fibrosis stages				
0	17.04 \pm 7.28	15.53 \pm 6.85	0.9	0.4
1	16.89 \pm 11.89	14.87 \pm 7.64	0.5	0.6
2	9.23 \pm 3.30	15.72 \pm 8.37	1.5	0.1
p-value	0.2	0.9		
Steatosis grades				
1	24.92 \pm 6.05	25.02 \pm 5.45	0.1	0.9
2	17.51 \pm 4.86	18.12 \pm 2.73	0.4	0.7
3	9.26 \pm 2.78	10.05 \pm 3.47	0.9	0.4
p-value	<0.001*	<0.001*		

NB: None of the studied patients had F3 or F4.

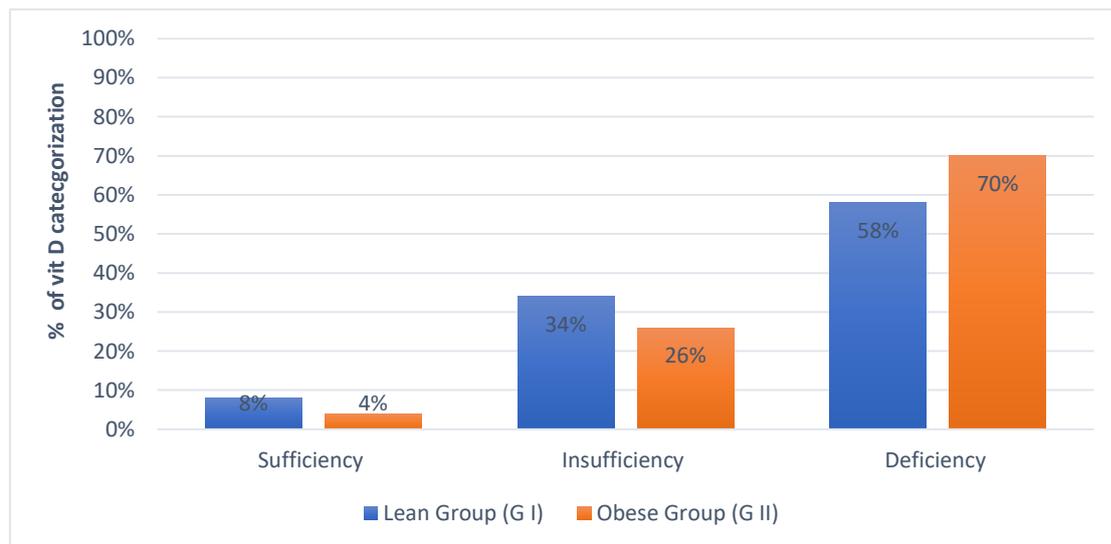


Figure (1): Comparison between the studied groups regarding serum levels of 25 hydroxy vitamin

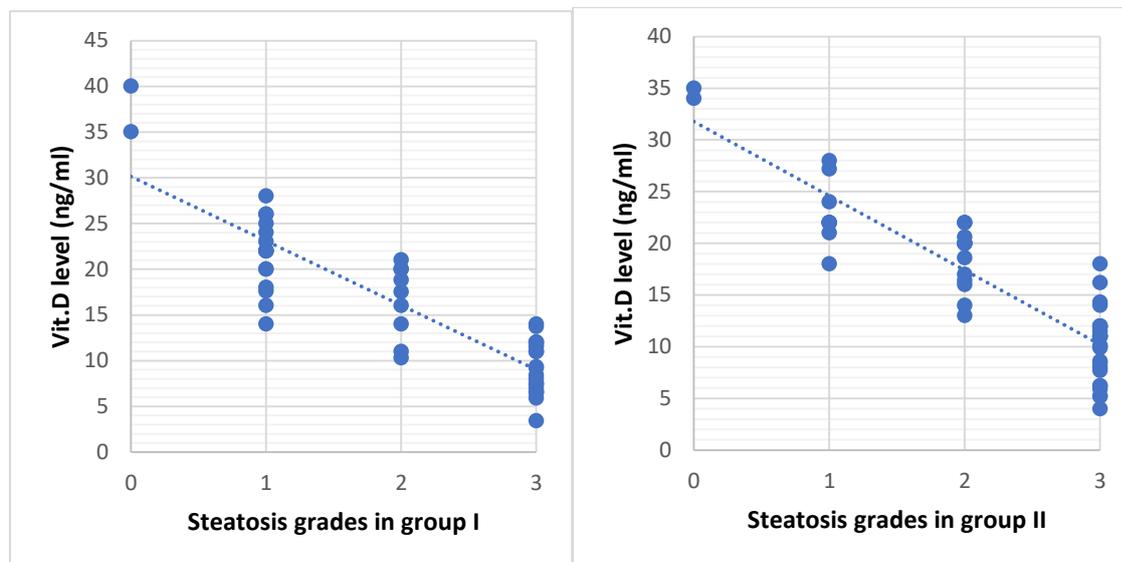


Figure (2): Correlation between Steatosis and 25 hydroxy Vit. D level in GI and GII

Males were predominant in G I (60%) while females were predominant in G II (62%) with statistically significant difference (**Table 1**). There was no statistically significant difference between the studied groups regarding routine laboratory findings (**Table 1**). The mean serum vitamin D level in GI was (16.38 ng/ml) while it was (15.44 ng/ml) in GII with no statistically significant difference (P-value =.6) (**Table 1**). Vitamin D

deficiency was more common in G II (70% vs 58.0% in GI) while insufficiency was more common in GI (34% vs 26% in GII). Sufficient vitamin D level was only found in 8% of GI compared to 4% of GII (**Figure 1**). There was no statistically significant difference between mean vitamin D level in different fibrosis stages in the studied groups and there was a statistically significant difference in both groups regarding the mean vitamin D level

in different steatosis stages (Table 2). Serum vitamin D level showed highly significant negative correlations with steatosis grades in both groups (P-value <0.001) (figure 2).

Discussion

In this study no statistically significant differences were detected between both groups regarding age with mean age of the lean group 41.16 ± 10.79 compared to 45.6 ± 11.99 of the obese group (P-value = 0.06), this was in agreement with who conducted their study on 2538 patients where there was no significant differences between lean and obese NAFLD groups regarding age (15).

In the study males were predominant in GI (60.0%) with a statistically significant difference between the two groups while females were predominant in G II (62.0%) (P-value = 0.03) it may be due to increase the risk of obesity in females in Egypt, this came in agreement with who reported that the prevalence of MAFLD was significantly higher in men (49.42%) than in women (27.97%) (16).

In the current study, there was no statistically significant difference between lean and obese MAFLD patient groups regarding 25 hydroxy vitamin D level (P-value=0.6). Serum vitamin D was low in both groups (16.38 ± 8.42 in GI vs 15.44 ± 7.21 in GII).

This comes in agreement with those who demonstrated that serum 25(OH)D₃ levels were inversely associated with NAFLD, even in subjects with normal body weight. (17).

In this investigated the role of 25(OH)D in NAFLD patients and matched the NAFLD group with a presumably healthy population that did not undergo liver ultrasonography (US). They found a strong inverse relationship between NAFLD and 25(OH)D levels (18).

Also, reported strong association between hypovitaminosis D and NAFLD which was independent on age, sex, BMI, lipid profile or glucose level (19).

A minor but significant difference in 25(OH)D levels between patients with and without NAFLD was found (20).

That was showed vitamin D deficiency was significantly related to NAFLD in men but not in women (21).

That stated adolescents with suspected NAFLD had significantly lower 25(OH)D levels than adolescents without suspected NAFLD (22).

Our results were in contrary with who found no significant differences between patients with NAFLD and those without NAFLD in serum vitamin D levels (23,24).

Another study proved that there is a significant correlation between serum vitamin D concentration and NAFLD in obese but not lean participants (15).

That stated the mechanisms by which 25(OH) vitamin D may induce NAFLD is not clear. The liver converts vitamin D to its active form, 25 (OH) vitamin D so in liver diseases the 25 (OH) vitamin D level is low (25).

This was found that vitamin D deficiency may induce NAFLD by impairing hepatic lipid metabolism⁽²⁶⁾.

That was demonstrated Patients with vitamin D deficiency found to have high rates of insulin resistance, metabolic syndrome and inflammatory mediators including IL-4, IL-6 and TNF- α ⁽²⁷⁾.

It was also reported that vitamin D receptors widely exist in liver tissue with negative association between vitamin D receptors expression and necro-inflammatory grades of NASH⁽²⁸⁾.

However, it was stated that Vitamin D may be sequestered in the adipose tissue in obese patients⁽²⁹⁾.

These contradictory results among studies may be related to differences in the studied population, nutritional, genetic, and environmental factors.

In the current study Vitamin D deficiency was more common in the obese group (70% vs 58.0% in GI) and vitamin D insufficiencies were more common in the Lean group (8% compared to 4% of GII).

This disagrees with who reported that Vitamin D deficiency and insufficiency were more common in the obese group than the lean group and in the obese group, they found that participants with vitamin D deficiency had the highest prevalence of NAFLD (57.60%), followed by those with vitamin D insufficiency (55.73%), and then those with vitamin D sufficiency (43.23%) ($P < 0.001$). In the lean group, the prevalence of NAFLD was comparable among participants with vitamin D

deficiency and those with vitamin D sufficiency (11.14% versus 10.89%)⁽³⁰⁾.

This difference may be due to small sample size used in our study. Our study revealed that vitamin D deficiency was more common in females than males in both groups with a statistically significant difference in the lean group (P Value=0.0001). This matched with who reported that women with NAFLD, compared to men with NAFLD, had significantly lower levels of 25(OH) D ($9.4 \pm 6.8 \mu\text{g/l}$ vs $13.6 \pm 7 \mu\text{g/l}$, $p < 0.0001$)⁽³¹⁾.

It was also reported that vitamin D deficiency was greater in females (46.9%) than in males (41.7%)⁽³²⁾. But this disagrees with who reported that Participants with higher 25(OH)D levels were more commonly females⁽³³⁾.

Our study revealed that vitamin D deficiency was more common with higher grades of fibrosis in G I. This came in agreement with who reported that vitamin D status was inversely correlated to Liver fibrosis by fibroscan. In the biopsy proven NAFLD patients⁽³⁴⁾.

Also, demonstrated an inversely association of advanced LF with vitamin D level⁽³⁵⁾.

However, revealed that advanced fibrosis identified by non-invasive scores are not connected to the low 25(OH)D in serum⁽³⁶⁾.

The inconsistent conclusions may result from the different measurements of LF and various vitamin D concentrations. In this study, we found that the higher the grade of steatosis the lower the Vitamin D level.

This matched with those who reported that the prevalence of vitamin D sufficiency was significantly lower in the group with higher grade of steatosis⁽³⁷⁾.

But this disagrees with those who reported that serum vitamin D is not connected to the CAP-defined NAFLD⁽³⁴⁾.

It was found that no significant association of the reduced vitamin D with the hepatic steatosis was found in general Portuguese population⁽³⁸⁾.

These different results may be due to different methods used to measure hepatic steatosis and various vitamin D concentrations⁽³⁸⁾.

Quantified hepatic steatosis according to Hamaguchi's ultrasonographic score (steatosis defined by a score ≥ 2) and in⁽³⁵⁾ study Participants were categorized as having either vitamin D deficiency (<50 nmol/L) or vitamin D sufficiency (≥ 50 nmol/L), unlike our study.

This study still has some limitations. First, the small sample size which might limit generalization of results. Second, We did not perform liver biopsy (the gold standard) or either magnetic resonance proton density fat fraction (MRI-PDFF) or magnetic resonance spectroscopy (MRS) because of invasiveness with subsequent complications and financial limitations respectively. Third, our study is a cross-sectional study, and further prospective studies are needed to analyse the causal relationship between vitamin D deficiency and progression of MAFLD, as well as prognostic effect of vit D supplementations on MAFLD patients.

Conclusions

MAFLD patients, whether lean or obese, show low serum vitamin D levels, which negatively correlate with steatosis grades.

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