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Prevalence and Possible Risk Factors of Metabolic Associated Fatty Liver Disease (MAFLD) in Non-Obese Individuals in El-Minia Governorate –Egypt

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ABSTRACT

Background: Obesity is a significant risk factor for metabolic associated fatty liver disease (MAFLD). It influences around 20%-30% of population worldwide and enhances the risk for hepatic and extra-hepatic complications. However, MAFLD occurs in lean subjects.

Objective: This study aimed to detect the prevalence of non-obese NAFLD in our community, to compare the socio-demographics, clinical and metabolic characteristics of non-obese versus obese MAFLD individuals, and to determine risk factors for MAFLD in non-obese subjects.

Materials and methods: 100 adults aged ≥18 years with BMI ≤ 25 were studied. In this study, clinical assessment, anthropometric measurements, laboratory tests, ultrasonography (US) and shear wave elastography were done.

Results: Among 100 subjects, 26 were MAFLD lean, while 74 healthy controls, the lean MAFLD females were 19 (73.1%), while 7 (26.9%) were males. Lean MAFLD was more common in females, housewives with possible risk factors like insulin resistance and hyperlipidemia**.**

Conclusion: MAFLD is present in non-obese individuals with possible risk factors like insulin resistance and hyperlipidemia.

Keywords: Dyslipidemia, Insulin resistance, Lean metabolic associated fatty liver disease.

INTRODUCTION

MAFLD (also known as non-alcoholic fatty liver disease) is a multisystem condition that has significant consequences, because it enhances the risk of hepatic cirrhosis, hepatic failure as well as hepatocellular carcinoma [(1, 2)]. It also can be associated with several extra-hepatic complications including cardiovascular diseases, chronic kidney diseases, diabetes [(3)], osteoporosis, and some types of malignancies [(4)].

There is a complex pathogenetic mechanism for MAFLD, which includes an interaction between environmental and genetic factors, with evidence of shared genetic factors between it and other metabolic disorders [(5)]. Though, usually, associated with obesity, a significant percentage of cases are non-obese, hence termed "lean MAFLD" [(6)]. The "lean NAFLD" was first recognized among Asian populations. However it also occurs in other ethnic groups, and might indicate visceral obesity without the presence of systemic obesity. Lean MAFLD develops in those with a normal BMI with cutoffs of 25 kg/m^2 and 23 kg/m^2 in Caucasian and Asian cases, respectively [(4)].

Recently, an international consensus advocated new diagnostic criteria for MAFLD, which are based on evidence of fatty liver, along with one of the following: overweight/obesity, diabetes, or dysregulated metabolism [(2)].

Our study aimed at detecting the prevalence of non-obese MAFLD in our community, comparing the sociodemographics, clinical and metabolic characteristics between non-obese and obese MAFLD subjects, and determining the risk factors for MAFLD in non-obese subjects.

PATIENTS AND METHODS

This study is an observational cross-sectional study, which was performed in the period from May to December, 2021. This study included 100 patients enrolled from the outpatient clinic of tropical medicine department at Minia University Hospital. The diagnosis of MAFLD was established based on the ultrasound characteristics, existence of insulin resistance (IR), or metabolic syndrome features, and histological confirmation whenever possible. MAFLD with BMI < 23 kg/m^2 were defined as lean MAFLD.

Exclusion criteria

Patients aged <18 years and evidence of other hepatic illnesses, e.g. alcoholic, viral hepatitis, autoimmune hepatitis, hereditary liver diseases, decompensated cirrhosis, malignancy, and alternative causes of fatty liver (e.g., on amiodarone) and congestive hepatopathy.

For comparing characteristics of lean MAFLD patients, all patients were ≥ 18 years and both sexes were included.

Patient's assessment and procedure:

Each patient was subjected to detailed history taking and examination (including anthropometric measurements) at the first visit. Anthropometric measurements included weight, height, BMI, waist circumference (WC), hip circumference (HC) and waist-hip ratio (WHR).

Following an overnight fasting, blood samples were obtained for laboratory tests including liver function tests, lipid panel as well as fasting serum insulin and

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blood sugar. Also, serum samples were tested for markers of viral hepatitis A, B, C, and E. Serum ferritin level, thyroid profile, and autoantibody tests. IR was measured as homeostasis model assessment insulin resistance (HOMA-IR), (HOMA-IR > 2.0 indicated the presence of IR) [7]. Socio-demographics included age, gender, occupation, marital state and smoking**.**

Laboratory tests:

A sample (5 mL venous blood) was collected from the fasting person with utilizing sterile syringe. Sera underwent separation and were stored at -20 °C till analysis. Blood sugar, alanine aminotransferase (ALT) triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) were measured using automatic biochemistry analyzer (Roche, Rotkreuz, Switzerland). Thyroid function tests and HOMA-IR were measured using ELISA.

1- Hepatic steatosis index (HSI score)**:** Was used for the scoring model for NAFLD/MAFLD (The cutoff value was settled as ≥ 36).

2-APRI score (AST to platelets ratio index)

- An APRI score > 0.7 has 77% sensitivity and 72% specificity for the prediction of severe liver fibrosis.

3-Fiborsis-4 (FIB-4) score was measured and was interpreted as follows:
• < 1.45 rules out fibrosis.
• > 1.45 and < 3.25 means mild- to-moderate (F1–F2) fibrosis.
• > 3.25 means significant fibrosis (F3–F4).

4- NAFLD Fibrosis Score (NFS): was used to distinguish between patients with NAFLD who have (F3-F4) and (F0-F2)

5- Calculation of HOMA-IR: Generally, optimum insulin sensitivity if the HOMA-IR is <1. Levels > 1.9 signal early IR, whereas levels > 2.9 signal significant IR [7].

6- WC and WHR were calculated.

Imaging Methodology:

- Ultrasonography

Hepatic US was performed for each participant, utilizing a high-resolution B-mode. The following findings were assessed: **(1)** Diffuse hyperechoic liver relative to the right kidney, **(2)** Attenuation of the US beam and (**3)** Poor visualization of intra-hepatic vasculature wall and architectural details.

- 2D-Shear Wave Elastography (SWE):

The same US system and convex probe was used for 2D SWE. The transducer was placed inter-costally at right lobe, with the targeted area located in the right anterior hepatic segment at an ideal depth of 3 cm to 5 cm from liver capsule (at least 1 cm depth). The major blood vessels were avoided. The left hepatic lobe was avoided as the measurement will be affected by cardiac movement.

Ethical approval:

This study followed the Helsinki Declaration and obtained its approval from the Faculty of Medicine, Minia University' Research Ethical committee. All patients provided informed written consents.

Statistical analysis

Data underwent analysis by IBM SPSS Statistics for Windows, V.24. Descriptive data were used for parametric quantitative data by means, standard deviation (SD) and minimum & maximum of the range, and for non-parametric quantitative data by medians, whereas they were expressed for categorical data as numbers and percentages. Analysis was performed for parametric quantitative data between the groups by independent t -test, and for non-parametric quantitative data by Mann Whitney test. Analysis was performed for qualitative data by Chi square test (if < 20% of cells have expected count < 5) or Fisher's Exact test (if >20% of cells have expected count < 5). Correlation between variables was done utilizing Spearman's rho correlation coefficient. ROC (Receiver Operating Characteristic) curve was performed to determine AUC, optimal cut-off point, sensitivity, PPV, NPV, and accuracy. P value ≤ 0.05 was considered statistically significant.

RESULTS

Table (1) showed that the mean age was 27.35 ± 10.04 in non-MAFLD, while it was 37.17 ± 16.20 in MAFLD group, which was statistically significant (P=0.007). As regards gender, males in non- MAFLD group were 39 (52.7%) versus 35 (47.3%) were females, while in MAFLD group males were 7 (26.9%) versus 19 (73.1%) were females and such difference was significant (P= 0.02). A higher prevalence of smoking was found among MAFLD cases (15.4%) than in non-MAFLD cases (8.1%), (P= 0.2).

Table (1): Demographics of the studied populations

Characteristic	Non-MAFLD (n =74)	MAFLD cases (n =26)	p- value
Age (years)			0.007*
Mean ± SD	27.35 ± 10.04	37.12 ± 16.20	
Sex			0.023*
Males	39(52.7%)	7(26.9%)	
Females	35(47.3%)	19(73.1%)	
Occupation			<0.0001*
Nurse	7(9.5%)	1(3.8%)	
Doctor	8(10.8%)	0(0.0%)	
Worker	29(39.2%)	4(15.4%)	
Student	13(17.6%)	1(3.8%)	
House wife	16(21.6%)	16(61.5%)	
Farmer	0(0.0%)	4(15.4%)	
Teacher	1(1.4%)	0(0.0%)	
Marital status			
Single	49(66.2%)	7(26.9%)	0.001*
Married	25(33.8%)	19(73.1%)	
Smoking			
Positive	6(8.1%)	4(15.4%)	0.281
Negative	68(91.9%)	22(84.6%)	

Chi square test (if < 20% of cells have expected count < 5) or Fisher's Exact test (if >20% of cells have expected count < 5) for qualitative data among groups. Independent T-test for quantitative data between the groups. P < 0.05 was considered statistically significant.

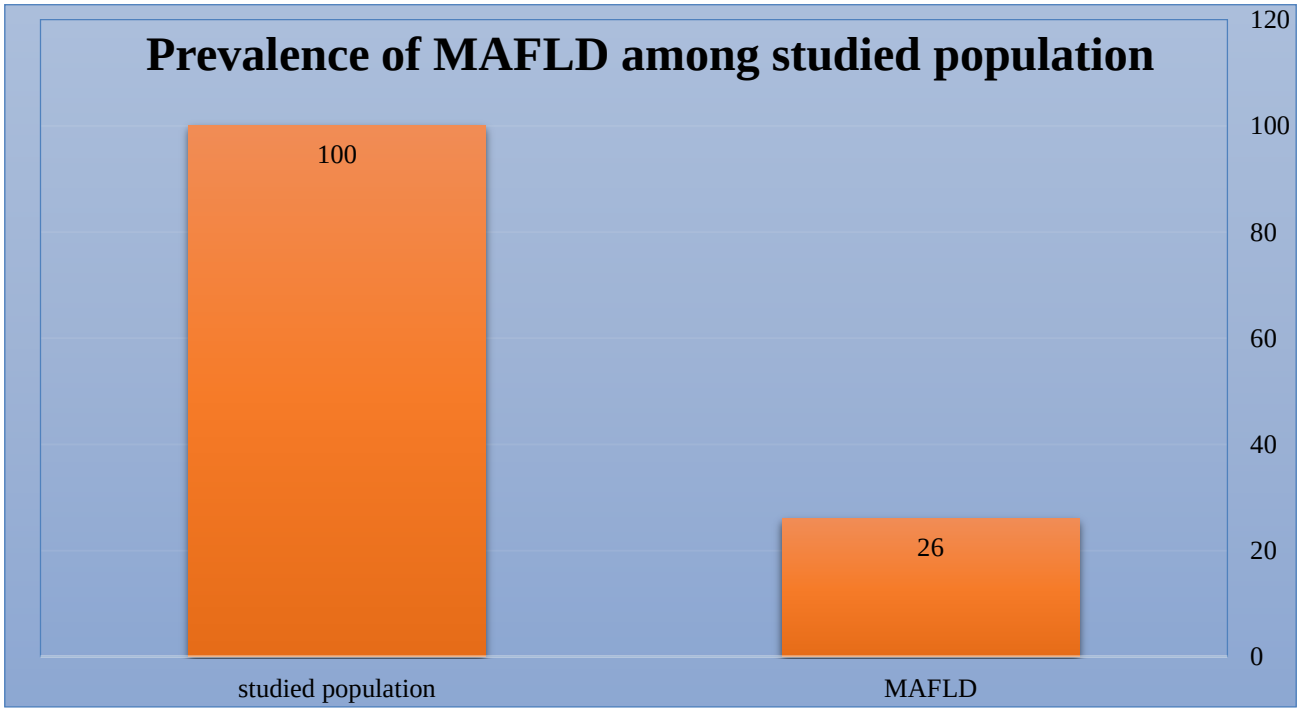

Figure (1): Prevalence of MAFLD in the studied population

Table (2) showed anthropometric measurements of the studied subjects. The study data showed that waist circumference in non-MAFLD was 81.08 ± 8.15 versus 87.00 ± 8.17 in MAFLD (P= 0.002). Waist/hip ratio was 0.84 ± 0.06 in non-MAFLD group versus (0.89±0.72) in MAFLD group (P=0.001).

Table (2): Anthropometric measurements among the studied population

Characteristic	Non-MAFLD cases (n =74)	MAFLD cases (n =26)	p- value
Weight (Kg)	60.08 ± 9.21	58.34 ± 8.45	0.401
Height (Cm)	164.68 ± 9.56	160.50 ± 7.77	0.031*
BMI	21.89 ± 2.27	22.54 ± 1.86	0.192
WC (Cm)	81.08 ± 8.15	87.00 ± 8.17	0.002*
HC (Cm)	96.17 ± 6.77	98.50 ± 6.42	0.130
WHR	0.84 ± 0.06	0.89 ± 0.72	0.001*

Data are presented as means ± SD Independent T-test test for quantitative data among the groups. P < 0.05 was considered statistically significant. WC: waist circumference, HC: hip circumference, WHR: waist-hip ratio, BMI: body mass index.

Table (3) showed that a significant difference was found among both groups regarding the lipid panel (TC and LDL) where cholesterol was 158.26 ± 35.08 in non-MAFLD group versus 171.61± 25.02 in MAFLD group with P value of 0.040. LDL was 99.39 ± 24.12 versus 111.77 ± 18.16 (P= 0.038). Also, fasting glucose, fasting insulin and HbA1c showed significant difference between the two groups (P < 0.0001) where mean was 5.85 ± 0.97 in non-MAFLD subjects vs 7.44 ± 1.61 in MAFLD subjects. Also, HOMA-IRI was 1.1 ± 0.26 versus 1.7 ± 0.41 with significant difference between both groups (P = 0.05).

Table (3): Laboratory parameters of the studied population

Characteristic	Non MAFLD cases (n =74)	MAFLD cases (n =26)	p- value
Hemoglobin (gm /dl)	13.83 ± 1.59	13.26 ± 1.89	0.146
TLC *1000	6.21 ± 1.51	6.36 ± 1.55	0.785
Platelets *1000	249.73 ± 53.73	262.04 ± 65.42	0.348
PC %	90.67 ± 9.54	93.42 ± 7.55	0.187
INR	1.07 ± 0.08	1.05 ± 0.06	0.192
Urea (mg/dl)	28.03 ± 6.81	28.38 ± 6.92	0.893
Creatinine (mg/dl)	0.78 ± 0.18	0.82 ± 0.18	0.420
ALT (U/L)	19.79 ± 4.88	19.38 ± 4.72	0.874
AST (U/L)	22.58 ± 5.54	18.46 ± 4.56	0.474
Total bilirubin (U/L)	0.49 ± 0.12	0.48 ± 0.11	0.819
Direct bilirubin (U/L)	0.15 ± 0.03	0.15 ± 0.03	0.819
Albumin (gm/dl)	5.03 ± 0.31	4.98 ± 0.39	0.483
Cholesterol (mg/dl)	158.26 ± 35.08	171.61 ± 25.02	0.040*
TG (mg/dl)	105.72 ± 25.32	109.46 ± 26.41	0.600
LDL (mg/dl)	99.39 ± 24.12	111.77 ± 18.16	0.038*
HDL (mg/dl)	38.95 ± 8.35	37.34 ± 6.49	0.378
Fasting Glucose (mg/dl)	92.15 ± 12.59	92.54 ± 14.32	0.896
Fasting Insulin (mIU/L)	5.85 ± 0.97	7.44 ± 1.61	0.05*
HbA1C %	5.39 ± 0.42	5.79 ± 0.52	<0.0001*
TSH (ng/ml)	2.11 ± 0.51	1.84 ± 0.44	0.240
T3 (ng/ml)	1.03 ± 0.24	1.00 ± 0.48	0.902
T4 (ng/ml)	9.24 ± 2.21	10.06 ± 2.47	0.350
HOMA IRI	1.1 ± 0.26	1.7 ± 0.41	0.05*

Data are expressed as means ± SDs. Independent T-test test for quantitative data between the groups. Significant level at P < 0.05

Table (4) on binary logistic regression analysis correcting for age, gender, and WC, lean MAFLD individuals showed a greater risk with metabolic syndrome. Where this association was significant for dyslipidemia (OR 1.52, P= 0.05) and dysglycemia (OR 1.57, P = 0.017).

Table (4): Binary logistic regression analysis for the prediction of MAFLD cases

Predictor	OR	p- value
Age	2.305	0.999
Gender • **Male** • **Female**	Reference 0.000	1.000
Smoking • **No** • **Yes**	Reference 0.000	1.000
Weight	0.243	1.000
Length	1.617	1.000
BMI	126.485	1.000
Waist circumference	0.843	1.000
Hip circumference	1.062	1.000
Cholesterol	0.215	0.998
TG	1.52	0.017
LDL	5.419	0.998
Fasting insulin	89.664	0.05
FIB.4score	0.000	1.000
NFS	0.072	1.000
APRI score	12314845521438.902	1.000
HOMA.IRI	0.000	0.999
Dysglycemia	1.57	0.017
Severity of US • **Normal** • **Mild** • **Moderate**	Reference 0.000 0.008	0.999 1.000

Figure (2) & table (5) showed ROC curve analysis of shear wave elastography grading in prediction of fibrosis among the studied population at baseline. For prediction of presence of fibrosis, adopting cutoff point of 0.65 m/s for elastography, the sensitivity and specificity were 76.9%, and 91.9% respectively for shear wave elastography in prediction of presence of fibrosis in MAFLD cases.

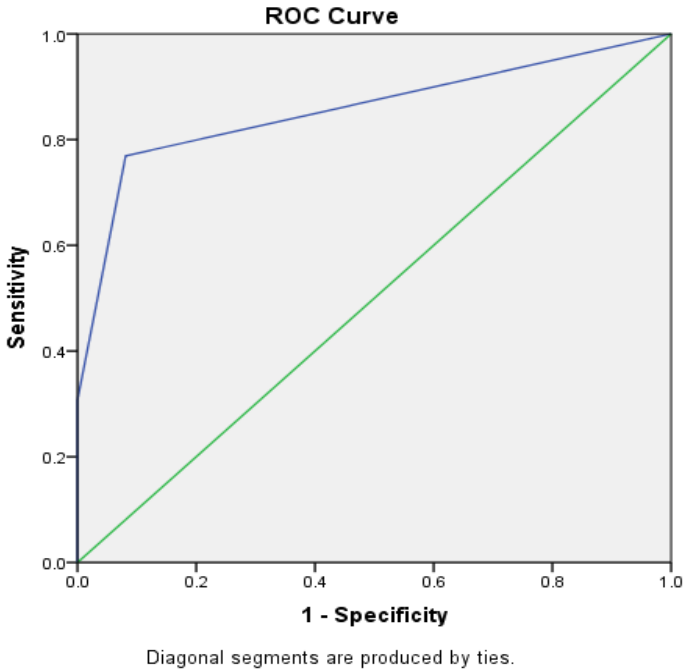

Figure (2): Roc curve of shear wave elastography grading in prediction of fibrosis among the studied population at baseline.

Table (5): ROC analysis of elastography grading in prediction of presence MAFLD

EST	AUC	Cut-off point	Sensitivity	specificity	Positive predictive	Negative predictive
Elastography	0.857	0.65 m/s	76.9%	91.9%	76.9%	91.9%

DISCUSSION

The prevalence of MAFLD among non-obese/lean MAFLD was approximately 26% (n= 26) of total studied population in our study (n= 100). This is consistent with **Alam *et al.*** [8] who found comparable results where their study included 465 cases, 119 were non-obese MAFLD (25.6. While, **Ito *et al.*** [9] found in their meta-analysis on 7752 MAFLD and 7135 non-MAFLD that only about 26% of MAFLD population in Japan were obese, around 21% were lean, while 53% were overweight.

In our study, when we compared non-MAFLD group with MAFLD group, we found that the mean BMI of non-obese MAFLD individuals was higher versus the BMI of non-obese individuals without MAFLD and the mean waist circumference of individuals with non-obese MAFLD was higher compared with that of non-obese individuals without MAFLD (87.00 ± 8.17 vs 81.08 ± 8.15) (P, 0.002). **Rahman *et al.*** [10] reported that the mean BMI of non-obese MAFLD individuals was higher compared to BMI of non-obese individuals with no MAFLD.

Dyslipidemia was more prevalent in lean MAFLD group than in lean non-MAFLD group with higher levels of cholesterol (171.61 ± 25.02) vs (158.26 ± 35.08 mg/dl), (p = 0.040), LDL (111.77 ± 18.16 vs 99.39 ± 24.12 mg/dl, p = 0.038) and lower levels of HDL-cholesterol (37.34 ± 6.49 vs 38.95 ± 8.35 mg/dl).

Also, dysglycemia was more common among lean MAFLD in comparison with lean without MAFLD because lean cases with MAFLD had a greater prevalence of prediabetes. The mean HbA1c of non-obese MAFLD cases was higher compared to non-obese individuals with no MAFLD (5.79 ± 0.52 vs 5.39 ± 0.42, p < 0.0001). This agrees with **Semmler *et al.*** [11] study in which 3,043 individuals (group I) and 1,048 individuals (group II) underwent screening investigations between 2010 and 2020. They were allocated into one of the subsequent groups: lean individuals with no MAFLD, lean MAFLD, overweight MAFLD (BMI 25–30 kg/m^2), and obese NAFLD (BMI >30 kg/m^2). MAFLD diagnosis was made by US in group I and controlled attenuation parameter (CAP) in group II, who found the same results except dyslipidemia with higher level of triglycerides not cholesterol [11].

We also found that non-obese/lean MAFLD had greater levels of fasting insulin and HOMA-IR as compared to non-obese participants without MAFLD. This is consistent with **Feng *et al.*** [12] study, at which 1779 subjects (134 lean MAFLD, 597 lean control and 1048 over weight) were included in this study where found similar results.

Fibroscan with CAP is an US based technology, which measures the ultrasound attenuation depending upon liver viscosity and the distance of propagation of US signals into the liver [13]. The stages of fibrosis in this study were as follows: F2: ≥ 1.76, F3: ≥ 2.21, and F4: ≥ 2.86 all were defined in m/s[233]. Most of cases had no or mild hepatic fibrosis [F0: 6 (23.1%), F1:12 (46.2%)], whereas 8 cases had moderate fibrosis [F2:8 (30.8%)], and no patient had severe fibrosis (F3, F4).

This study revealed a significant relationship between Cholesterol, TG, LDL, and LSM measured by 2D-SWE. This agrees with **Fabrellas *et al.*** [14] study on 215 individuals with metabolic risk factors with no liver disease. Eighty individuals were age- and sex-matched and had no metabolic risk factors and served as control group. LSM were evaluated by TE and a good significant association was revealed between transaminases and increased LSM, suggesting hepatic fibrosis.

LIMITATIONS

The small number of the studied population. MAFLD patients did not undergo liver biopsy in our study. Liver biopsy is still the gold standard for fibrosis staging, however it is unsuitable for general use due to its invasive nature. Moreover, 2D-SWE only does not allow quantitative assessment of hepatic steatosis. The possibility of simultaneous evaluation of hepatic fibrosis (utilizing LSM) and steatosis (utilizing CAP) makes US elastography a valuable non-invasive method for the evaluation and the quantification of steatosis and fibrosis among MAFLD cases.

CONCLUSION

MAFLD is common in non-obese subjects. Our study revealed that the prevalence of MAFLD among lean individuals was about 26% among the studied population. Also, we found that non-obese MAFLD has higher BMI, waist circumference, dyslipidemia, dysglycemia and HOMA-IRI than non-obese subjects without MAFLD.

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