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Serum Nesfatin-1 in Patients with Metabolic Associated Fatty Liver Disease

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ABSTRACT
Background: Metabolic associated fatty liver disease (MAFLD), formerly known as nonalcoholic fatty liver disease (NAFLD), is a condition characterized by the accumulation of fat in the liver, which can lead to inflammation, scarring, and liver damage. **This study aimed to** assess serum nesfatin-1 levels in patients diagnosed with MAFLD. **Patients and Methods:** This case-control study included 76 participants, implemented at Gastroenterology and Hepatology Unit, and Outpatient Clinic of Internal Medicine Department of Benha University Hospital during the period from April to September 2023. They were divided into two equal groups: Group I: included 38 patients with MAFLD, while group II: included 38 apparently healthy age and sex matched individuals as a control group. **Results**: Serum nesfatin-1 showed a significant negative correlation with alanine aminotransferase (ALT), and it showed a significant negative correlation with high sensitivity-C reactive protein (hs-CRP) and fasting insulin level in the MAFLD group. Logistic regression analysis was conducted for prediction of MAFLD revealing that aspartate aminotransferase (AST), ALT, γ-glutamyl transferase (GGT), hs-CRP, high density lipoprotein (HDL), low density lipoprotein (LDL), fasting insulin, homeostatic model assessment insulin resistance (HOMA-IR) and nesfatin-1were all associated with the risk of MAFLD in univariate analysis. A receiver operating characteristic (ROC) curve of nesfatin-1 was conducted for prediction of MAFLD, and it showed moderate accuracy with an area under curve (AUC)=0.776 at best cut-off value of 0.270, with 86.8% sensitivity and 84.2% specificity. **Conclusion:** Nesfatin-1 may be a potential biomarker for the diagnosis of MAFLD.
Keywords: Serum Nesfatin-1; Metabolic Associated Fatty Liver Disease; Non-alcoholic Fatty Liver Disease; Metabolic Dysregulation; Nucleobindin-2.

INTRODUCTION

NAFLD is one of the most common chronic liver diseases worldwide. It has a wide spectrum range from simple steatosis to non-alcoholic steatohepatitis and cirrhosis. It is closely associated with metabolic syndrome, insulin resistance, and obesity. The presence of metabolic disorders and hepatic fibrosis both lead to adverse outcome in patients with NAFLD [1].

MAFLD is a novel concept proposed in 2020 aiming to replace the term NAFLD**.** Unlike NAFLD, MAFLD does not require the exclusion of other etiologies of liver disease, such as excessive alcohol consumption or viral hepatitis. MAFLD is diagnosed in patients when they have both hepatic steatosis and any of the following three metabolic conditions: overweight/obesity, diabetes mellitus, or evidence of metabolic dysregulation (MD) in lean individuals**.** This novel concept and criteria enable clinicians to identify more patients at risk of adverse outcomes in clinical practice [2].

Nesfatin-1 is a novel peptide of 82 amino acids, that is encoded by the nucleobindin-2 (NUCB2) gene and defined as the satiety peptide associated with melanocortin signaling in the hypothalamus [3].

Nesfatin-1 is present in paraventricular nucleus, lateral hypothalamic area, supraoptic nucleus, dorsomedial nucleus and arcuate nucleus of the hypothalamus, solitary tract nucleus, and some peripheral tissues (e.g., adipose tissue). It is a molecule associated with dietary habits and has an anorexigenic action. Following a 24-hour fasting, expression of nesfatin-1/ NUCB2 gene and concentration of nesfatin-1 in the paraventricular nucleus of the hypothalamus is decreased. Studies demonstrate that centrally or peripherally applied nesfatin-1 depresses food intake, because of that inhibiting weight gain. Also, intravenous nesfatin-1 administration was shown to decrease glucose level in mice [4].

Insulin resistance has been reported to be the major key mechanism involved in the pathogenesis of NAFLD. Molecular and animal studies suggested nesfatin-1 beneficial effects on glucose and lipid metabolism as it augments insulin. It regulates energy homeostasis via its central anorexigenic effect and decreased body weight effect. Interestingly, some evidence revealed the regulatory effect of nesfatin-1 on adipogenesis [5].

Few contradictory data were found about the relationship between circulating levels of nesfatin-1 and NAFLD. While reported reduced serum levels of nesfatin-1 in patients having NAFLD, another study revealed increased plasma levels of nesfatin-1 in rat models of NAFLD [6,7]**.**

The purpose of this study was to assess serum nesfatin-1 levels in patients diagnosed with MAFLD.

PATIENTS AND METHODS

This case-control study included 76 participants, implemented at Gastroenterology and Hepatology Unit and Outpatient Clinics of Internal Medicine Department of Benha University Hospital. The study was performed during the period from April to September 2023

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Exclusion criteria were patients with malignancy, organ transplantation, prior abdominal surgery, decompensated liver cirrhosis or hepatocellular carcinoma, patients with serious illness (like acute diabetic complications, acute heart failure, sepsis, overt kidney disease), patients on steroids, estrogen, amiodarone, tamoxifen or lipid-lowering drugs, and finally pregnant and lactating females were excluded.

The studied subjects were divided into 2 groups: Group I: included 38 patients with MAFLD; and group II: included 38 apparently healthy age and sex matched individuals as a control group.

Methods

All participants were subjected to detailed history taking with special emphasis on demographic variables (age, gender, life style factors as physical activity, smoking, and alcohol consumption), history of diabetes mellitus and hypertension, presence of comorbidities, drug and surgical history, and thorough clinical examination including: Blood pressure measurement, performed in a quiet room by using a sphygmomanometer after 10 minutes of rest, an anthropometric measures including (weight that was measured by body weight scale in light clothing without shoes, and height that was measured by a measuring tape). Calculation of body mass index (BMI), and laboratory biochemical investigations including complete blood count (CBC), ALT, AST, total bilirubin, GGT, alkaline phosphatase (ALP), fasting plasma glucose, fasting plasma insulin, HbA1c, urea and creatinine, and lipid profile; total cholesterol, HDL, LDL, and triglycerides. Insulin resistance (IR) was determined by using HOMA-IR = [fasting blood glucose (mg/dL) × fasting plasma insulin (μU/mL)] / 405 [6], and hs-CRP.

The quantitative determination of serum nesfatin-1 levels: Venous blood samples (total of 2 mL blood) were drawn from the participants in the morning after (≈12-14 hours) fasting. The serum samples of each participant were collected into a vacutainer serum separator tube and separated into clean dried Eppendorf tubes and stored at −80°C until needed. The concentration of nesfatin-1 in serum was determined using a Human Nesfatin-1 ELISA kit (Phoenix Pharmaceuticals, Inc., USA).

Abdominal ultrasonography (US): It was performed by an experienced and trained physician to confirm the diagnosis of fatty liver as (diffuse hyperechoic echotexture, deep attenuation, increased liver echotexture compared with the kidney, and vascular blurring [7]. According to the severity of liver steatosis in the US, patients were further classified as having mild, moderate, or severe liver steatosis. The grading of liver steatosis was done by using features that include liver brightness, contrast between the liver and kidney, ultrasonographic appearance of the intrahepatic vessels, liver parenchyma, and diaphragm [8].

Ethical approval:

The study was approved by Internal Medicine Department of Benha University Hospital (Approval code MS 15-2-2023) and by the Research Ethics Committee, Faculty of Medicine, Benha University. An informed written consent was obtained from the participants. Everyone received an explanation of the purpose of the study and had a secret code number. The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis

Statistical analysis was done by SPSS v26 (IBM Inc., Armonk, NY, USA). Quantitative variables were presented as mean and standard deviation (SD) and compared between the two groups utilizing unpaired Student's t- test. Qualitative variables were presented as frequency and percentage (%) and were analyzed utilizing the Chi-square test. Pearson correlation was used to estimate the degree of correlation between two quantitative variables. Logistic univariate regression was also used to estimate the relationship between a dependent variable and one or more independent variables. A two tailed P value < 0.05 was considered statistically significant.

RESULTS

Regarding demographic data of the studied subjects, age and gender were insignificantly different between the two studied groups. Waist circumference, BMI, SBP, and DBP were statistically significantly higher in MAFLD group than control group. Regarding laboratory investigations: AST, ALT, GGT, hs-CRP, triglycerides, HA1C, fasting blood sugar, fasting insulin and HOMA-IR were significantly higher in the MAFLD group than the control, however total cholesterol, HDL, LDL, and serum nesfatin-1 level were significantly lower in MAFLD group than the control. Alkaline phosphatase, total bilirubin, creatinine, and urea were insignificantly different between both studied groups **(Table 1)**.

Table 1: Demographic data, anthropometric measurements, and laboratory variables in the studied groups

		MAFLD n=38	Controls n=38	p
Demographic data and anthropometric measurements				
Age (years)		49.74±8.02	50.05±6.53	0.854
Gender n (%)	**Male**	25(65.8%)	24(63.2%)	0.811
	Female	13(34.2%)	14(36.8%)	
Waist circumference (cm)		99.12±10.79	88.43±9.19	**<0.001****
BMI (kg/m^2)		32.26±2.92	25.83±3.95	**<0.001***
SBP (mmHg)		136.42±4.88	119.88±3.7	**<0.001****
DBP (mmHg)		89.33±2.09	80.04±1.42	**<0.001****
Laboratory variables				
AST (IU/L)		41.05±5.94	25.98±4.20	**<0.001****
ALT (IU/L)		53.12±5.56	23.95±5.67	**<0.001****
GGT (IU/L)		47.66±3.25	22.37±4.18	**0.002***
Alkaline phosphatase (IU/L)		74.55±4.5	72.73±9.33	0.117
Total bilirubin (mg/dL)		0.76±0.18	0.71±0.17	0.285
Creatinine (mg/dL)		1.07±0.29	0.83±0.08	0.253
Urea (mg/dL)		19.49±2.18	21.93±2.36	**<0.001****
hs-CRP		2.97 ±0.03	1.11±0.4	**<0.001****
Lipid profile	**Total cholesterol (mg/dL)**	224.86±8.19	230.36±33.8	**0.006***
	Triglyceride (mg/dL)	154.71±7.99	115.28±25.26	**<0.001****
	HDL (mg/dL)	38.08±4.37	46.87±2.93	**0.029***
	LDL (mg/dL)	126.04±57.19	130.1±22.91	**0.005***
Hemoglobin A1c (%)		6.27±1.27	5.85±0.65	**0.048***
Fasting blood glucose (mg/dL)		124.99±5.49	99.78±14.44	**0.033***
Insulin (mg/dl)		17.56±3.79	9.69±2.41	**<0.001****
HOMA-IR		5.48±1.35	2.37±0.64	**<0.001****
Nesfatin-1 (ng/ml)		0.26±0.01	0.42±0.12	**0.002***

Data are presented as mean ±SD and number (%), BMI: body mass index, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: γ-glutamyl transferase, hs-CRP: hypersensitive C-reactive protein, HDL: high-density lipoprotein, LDL: low-density lipoprotein, HOMA-IR: homeostatic model assessment, *: P value <0.05 significant, **: P value< 0.01 is highly significant.

ROC curve of nesfatin-1 was conducted for prediction of MAFLD, and it showed moderate accuracy (AUC=0.776) at a cut-off value 0.270 with sensitivity of 86.8% and specificity of 84.2% **(Figure 1)**.

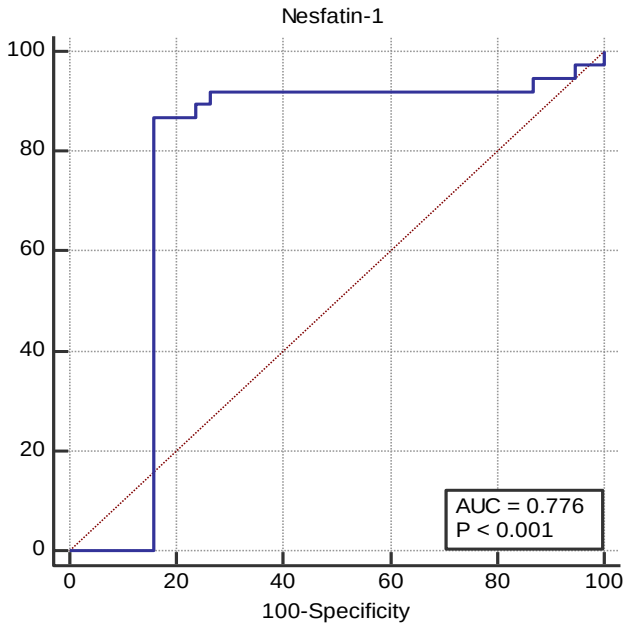

Figure 1: ROC curve of nesfatin-1 for prediction of MAFLD.

Nesfatin-1 showed a significant negative correlation with ALT, AST, hs-CRP and fasting insulin in the MAFLD patients (**Table 2)**.

Table 2: Correlation analysis between nesfatin-1 and other parameters in the MAFLD group

Variable	R	p
Age	-0.113	0.330
Waist circumference (cm)	-0.104	0.370
BMI (kg/m^2)	0.061	0.602
AST (IU/L)	-0.188	0.104
ALT IU/L	-0.337	**0.003***
GGT IU/L	0.131	0.259
Alkaline phosphatase (IU/L)	-0.105	0.366
Total bilirubin (mg/dL)	0.247	0.612
Creatinine (mg/dL)	-0.055	0.636
Urea (mg/dL)	-0.003	0.978
hs-CRP	-0.283	**0.013***
Total cholesterol (mg/dL)	0.101	0.384
Triglyceride (mg/dL)	-0.210	0.068
HDL (mg/dL)	0.136	0.242
LDL (mg/dL)	0.123	0.288
Hemoglobin A1c (%)	0.109	0.349
Fasting blood glucose (mg/dL)	0.003	0.978
Insulin (mg/dL)	-0.227	**0.048***
HOMA-IR	-0.215	0.062

r: Correlation Coefficient, BMI: body mass index, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: γ-glutamyl transferase, hs-CRP: hypersensitive C-reactive protein, HDL: high-density lipoprotein, LDL: low-density lipoprotein, HOMA-IR: homeostatic model assessment, *: P value <0.05 significant.

Logistic regression analysis for the prediction of MAFLD showed that AST, ALT, GGT, hs-CRP, HDL, LDL, fasting insulin, HOMA-IR and nesfatin-1 were all associated with the risk of MAFLD in univariate analysis (**Table 3)**.

Table 3: Logistic univariable regression analysis for risk factors of MAFLD

	B	95% CI	p
AST (IU/L)	1.0387	1.006-1.071	**0.018***
ALT (IU/L)	1.0284	1.01-1.046	**0.002***
GGT (IU/L)	1.0230	1.007-1.039	**0.004***
hs-CRP	0.0002	0-0.046	**0.002***
Total cholesterol (mg/dL)	1.0015	0.994-1.008	0.697
Triglyceride (mg/dL)	0.9698	0.953-0.986	**<0.001 ****
HDL (mg/dL)	1.1073	1.04-1.177	**0.001***
LDL (mg/dL)	1.0022	0.991-1.012	0.682
Hemoglobin A1c (%)	0.8548	0.64-1.139	0.285
Insulin (md/dl)	0.5481	0.412-0.728	**<0.001 ****
HOMA-IR	0.1557	0.061-0.393	**<0.001 ****
Nesfatin-1 (ng/ml)	53379.9445	36.327-78437915.736	**0.003***

ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: γ-glutamyl transferase, hs-CRP: hypersensitive C-reactive protein, HDL: high-density lipoprotein, LDL: low-density lipoprotein, HOMA-IR: homeostatic model assessment, *: P value <0.05 significant, **: P value< 0.01 is highly significant.

DISCUSSION

MAFLD, formerly known as NAFLD, is a condition characterized by the accumulation of fat in the liver, which can lead to inflammation, scarring, and liver damage. MAFLD is commonly associated with obesity, type 2 diabetes mellitus (T2DM), dyslipidemia, and hypertension, and its prevalence is increasing worldwide, affecting about 25% of the global population. In addition to liver-related complications, MAFLD is associated with an increased risk of cardiovascular disease and mortality [9]. The aim of this study was to assess serum nesfatin-1 levels in patients diagnosed with MAFLD.

Age and gender were insignificantly different between both groups. Waist circumference, BMI, SBP and DBP were significantly higher in the MAFLD group than the control (P-value < 0.05).

The results of our study are consistent with several other studies that have reported similar demographic and anthropometric measurements in MAFLD patients. A study conducted by **Li *et al.*** [10] in China on 2868 adults found that the mean age of MAFLD patients was 50.5 years and that they had a significantly higher BMI and waist circumference compared to non-MAFLD individuals. Another study conducted by **Sookoian and Pirola** [11] in Argentina reported a higher prevalence of MAFLD among males and a significant association between MAFLD and central obesity. A study by **Huang *et al.*** [12] in Taiwan on 175 patients also found that MAFLD patients had higher BMI, waist circumference, and blood pressure compared to controls.

Regarding laboratory investigations, AST, ALT, GGT and hs-CRP were significantly higher in MAFLD group than the control one (P-value < 0.05). However, Alkaline phosphatase, total bilirubin, creatinine and urea were insignificantly different between both studied groups. Parallel to our finding, a cross-sectional study by **Kasapoglu *et al.*** [13] revealed that AST, ALT, and GGT levels were significantly higher in the MAFLD group compared to control ($p<0.05$). Additionally, a meta-analysis by **Mantovani *et al.*** [14] found that elevated ALT, AST, and GGT levels were associated with an increased risk of MAFLD.

According to lipid profile, total cholesterol, HDL and LDL were significantly lower in MAFLD group than the control (P-value < 0.05), while triglycerides was significantly higher in MAFLD group than the control (P-value < 0.05). According to blood sugar panel: HA1C, fasting blood sugar, fasting Insulin and HOMA-IR were significantly higher in MAFLD group than the control (P-value < 0.05). Supporting our findings, a systematic review and meta-analysis by **Martin *et al.*** [15] found that individuals with MAFLD had lower levels of total cholesterol, HDL and LDL, and higher levels of triglycerides compared to control group. Similarly, a study by **Ampuero *et al.*** [16] found that MAFLD patients had lower levels of total cholesterol and HDL compared to controls.

In addition, our results are consistent with previous studies that have reported an association between MAFLD and insulin resistance. A study by **Targher *et al.*** [17] found that MAFLD patients had significantly higher levels of fasting insulin and HOMA-IR compared to controls. Similarly, a study by **Buzzetti *et al.*** [18] found that MAFLD patients had higher levels of fasting glucose and insulin resistance compared to the control.

Furthermore, our study found that serum nesfatin-1 levels were significantly lower in the MAFLD group than the control group. ROC curve of nesfatin-1 was conducted to predict MAFLD, and it showed moderate accuracy with AUC of 0.776 at the best cut-off value of 0.270, with a sensitivity of 86.8% and specificity of 84.2%.

There have been several studies investigating the relationship between nesfatin-1 and MAFLD. A study by **Habib and Khalil *et al.*** [19] found that nesfatin-1 levels were significantly lower in MAFLD patients than healthy control, which is consistent with the findings in the current study. Furthermore, **Alotibi *et al.*** [20] found that nesfatin-1 levels were inversely correlated with liver fibrosis and steatosis scores in MAFLD patients. Similarly, another study by **Yang *et al.*** [21] found that nesfatin-1 levels were significantly lower in MAFLD patients compared to controls, and that there was a negative correlation between nesfatin-1 levels and the degree of hepatic steatosis. These findings suggest that nesfatin-1 may serve as a potential biomarker for the diagnosis and monitoring of MAFLD.

In the current study, nesfatin-1 showed a significant negative correlation with ALT, hs-CRP and fasting insulin in the MAFLD patients. Nesfatin-1 has been found to have a relationship with liver enzymes in various studies. For example, a study by **Yang *et al.*** [21] found that nesfatin-1 levels were significantly lower in NAFLD patients and were negatively correlated with ALT levels. Similarly, another study by **Alotibi *et al.*** [20] found a significant negative correlation between nesfatin-1 levels and ALT levels in patients with chronic hepatitis B.

In terms of the negative correlation between nesfatin-1 and hs-CRP, a comparative study by **García-Galiano *et al.*** [22] found that nesfatin-1 levels were negatively correlated with inflammatory markers such as CRP and tumor necrosis factor alpha (TNF-alpha). This suggests that nesfatin-1 may have anti-inflammatory effects.

Regarding the negative correlation between nesfatin-1 and fasting insulin, a study by **Mirakhor *et al.*** [23] found that nesfatin-1 levels were negatively correlated with insulin resistance in obese individuals.

In contrast to our study, in a study by **Kim *et al.*** [24] that prospectively included 78 of Korean children and adolescents (42 obese/overweight group and 36 healthy control group), serum nesfatin-1 levels were significantly lower in obese/overweight group than in control group (median 1.4 vs 2.0 ng/mL; P = 0.003). They reported that there was no association between serum nesfatin-1 and insulin resistance among obese children and adolescents.

Logistic regression analysis was conducted for the prediction of MAFLD and revealed that AST, ALT, GGT, hs-CRP, HDL, LDL, fasting insulin, HOMA-IR and nesfatin-1were all associated with the risk of MAFLD in univariate analysis.

Some trials have investigated the predictors of MAFLD, and their results are consistent with the current study. For instance, a cross-sectional study of Chinese cohort by **Zou *et al.*** [25] showed that AST, ALT, GGT, and hs-CRP were significantly associated with the risk of MAFLD.

Another study by **Feng *et al.*** [26] reported that low HDL and high triglycerides were independent risk factors for MAFLD. Moreover, fasting insulin and HOMA-IR have also been reported to be predictors of MAFLD in previous studies [12]. Lastly, our study also found that nesfatin-1 was associated with the risk of MAFLD in univariate analysis, which is consistent with the findings of a study by **Yang *et al***. [21], which reported that nesfatin-1 was an independent predictor of MAFLD**.**

CONCLUSION

Nesfatin-1 may be a potential biomarker for the diagnosis of MAFLD, as it showed a moderate accuracy with an AUC of 0.776 in predicting MAFLD. Logistic regression analysis also demonstrated that nesfatin-1 was associated with the risk of MAFLD.

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