Association Between Nesfatin-1 Levels and Obesity in Type 2 Diabetes Patients

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Abstract: One of the most serious consequences of obesity is Type 2 Diabetes mellitus. It has been linked to a chronic systemic inflammatory condition that plays a central role in insulin resistance associated with Type 2 Diabetes Mellitus. This study aimed to evaluate serum nesfatin-1 levels in patients with type 2 diabetes mellitus and to interpret these levels in relation to obesity, as measured by body mass index, and Homeostatic Model Assessment for Insulin Resistance). A total of 60 patients and 30 controls were recruited for this research. We assessed BMI, HOMA-IR, insulin levels, fasting plasma glucose. HbA1c, and serum nesfatin-1 concentrations for all participants. The serum insulin level (23.19 vs. 13.20 µIU/ml) and HOMA (13.50 vs. 2.12) were significantly elevated in patients compared to controls. Conversely, the nesfatin-1 level was notably lower in patients (55.12 ± 7.1 ng/ml vs. 79.03 ± 7.1 ng/ml). Receiver Operating Characteristic (ROC) analysis indicated significant variations in the following descending order: FPG (0.959), serum insulin (0.692), and serum nesfatin-1 (0.869). Furthermore, a significant negative correlation was observed between nesfatin-1 and obesity. When a multiple linear regression model was applied to examine the independent effects of BMI and HOMA, a significant negative correlation was found with nesfatin-1 levels. Additionally, the odds ratio (OR=33.3) indicated that the likelihood of having a high HOMA among patients was significantly increased with lower nesfatin-1 levels. In conclusion, lower nesfatin-1 levels are associated with a heightened risk of T2DM, particularly among obese individuals with diabetes.

1 INTRODUCTION

According to the World Health Organization (WHO), obesity is now the biggest chronic health concern globally, surpassing malnutrition as a significant health issue [1]. If current trends persist, it is projected that by 2030, 60% of the world's population will be overweight or obese [2], [3]. Obesity is a major risk factor for various noncommunicable diseases, including type 2 diabetes. The WHO states that overweight and obesity contribute to 44% of diabetes cases [4], [5]. The connection between obesity and type 2 diabetes (T2DM) is particularly strong, with the incidence of obesity-related diabetes expected to rise to 300 million cases by 2025 [6]. Oxidative stress and obesity have been linked. Patients who are obese have a higher chance of developing diabetes, suggesting a pathophysiological connection between oxidative stress, diabetes, and obesity [7]. The term "diabesity" has been coined to highlight this connection, as the majority of individuals with diabetes are either overweight or obese (International Diabetes Obesity increases an individual's risk of death by a factor of seven [8]. Nesfatin-1, a recently identified neuroendocrine peptide, plays a crucial role in maintaining energy balance and regulating food intake. Since the hypothalamus is primarily responsible for controlling hunger and satiety, nesfatin-1 has become a topic of increasing interest among researchers in obesity studies. Some findings have suggested that nesfatin-1 serves as an important inhibitory regulator of food intake and body weight [9]. Additionally, recent studies have indicated that nesfatin-1 may also be involved in the pathophysiology of diabetes mellitus (DM) [10]. However, most research on nesfatin-1 has been conducted in animal models, leaving its actual role in humans largely unknown [11]. Due to the lack

of information regarding the role of nesfatin-1 in both obesity and diabetes outside of animal studies, the present research aims to determine fasting serum nesfatin-1 levels in two groups (healthy and diabetic), including individuals who are normal-weight, overweight, and obese. These findings will be analyzed based on obesity (measured by BMI) and HOMA-IR (Homeostasis Model Assessment of Insulin Resistance).

2 MATERIALS AND METHODS

Anthropometric measurement, including age, weight, height, Body Mass Index (BMI) has been calculated according to a specific formula, which includes weight divided by the square of height, as the flaunting equation [12].

Several methods have been employed to assess insulin resistance (IR). The common one includes fasting insulin (μU/mL) and glucose (mg/dL) for calculating the homeostatic model assessment (HOMA), as shown in the equation below. The analysis of insulin resistance is crucial because this factor is implicated in the balance of several metabolic pathways [13].

HOMA-IR= [FBS (mg\dl) × FI (μU/ml)] /405.

2.1 T2DM Patients

Sixty diabetic patients (Iraqi Arabs), with an age range of 37-70 years. The diabetic patients had a mean age of (49.33±6.64) years. The cases that were excluded from this study include type I DM, metabolic syndrome, POC, pregnant women, smokers, liver disease, kidney disease, and hypertensives.

2.2 Healthy Subjects

Thirty healthy control subjects comparable to type 2 diabetes mellitus patients in respect to age (37-70 years) and from both sexes were presented in the study. The controls were selected according to physician criteria: Healthy with non -diabetic, not hypertensive, free of acute disease, with no history of alcohol drinking or smoking.

2.3 Sample Collection

Ten milliliters of blood have been collected from each patient and control. The samples were taken at the time between 8.00 and 11.00 A.M, after 12-15 hours of fasting. Two aliquots of the blood sample were taken. Ethylene diamine tetraacetic acid (EDTA) (1.5 mg/ml) has been used for the first part to estimate the HbA1c within less than three hours. While the second part was used for serum collection, the sample was dispensed into a plain tube to allow it to clot at room temperature (22 °C). Then, the serum was collected after centrifuging the sample at 3000 rpm. The collected serum was divided into several parts, each part contained 500μl in Eppendorf tubes and stored using a freezer (-20 °C) until use.

2.4 Determination of Insulin

Serum insulin levels were measured using a sandwich enzyme immunoassay method with a kit from Cusabio, China. In this technique, a specific insulin antibody is pre-applied to a microplate. When samples and standards are added, the immobilized antibody binds to the insulin present in the samples. After unbound materials are removed, the samples are treated with a specific conjugated antibody for insulin. Following a subsequent washing step, horseradish peroxidase is introduced. Another washing procedure is conducted to eliminate any unbound enzyme reagent. Once the substrate solution is added, the color of the final product develops, and the intensity of the color is measured after halting the development process.

2.5 Determination of Nesfatin-1

Using a kit provided by Biosource, USA, competitive enzyme immunoassay methods were employed to measure the levels of nesfatin-1. This experiment utilized a polyclonal anti-nesfatin-1 antibody and a nesfatin-1-HRP conjugate. Initially, the sample mixture, buffer, and nesfatin-1-HRP conjugate are incubated in a pre-coated plate for one hour. The next step involves decanting and washing the wells five times. Afterward, the HRP enzyme is added to the cleaned wells, and the samples are incubated together. A reaction occurs between the enzyme and the substrate, resulting in the formation of a blue-colored complex. To halt this reaction, a stop solution is added, which changes the color to yellow. The color intensity is subsequently measured using a microplate reader at 450 nm.

2.6 Statistical Methods

Data were entered into a database, and statistical analyses were conducted using SPSS version 25. The Kruskal-Wallis test assessed differences in medians among more than two groups. At the same time, normal distributions were found for BMI, FSG, and

HbA1c, with the independent t-test used for mean comparisons between the two groups. ROC analysis evaluated the effectiveness of parameters in distinguishing groups, indicating better discrimination as the area under the curve approaches one. A multiple linear regression model analysed the effects of BMI and the insulin resistance index (HOMA) on selected outcomes, with nesfatin-1 log-transformed for normalisation. This model highlighted the relative contributions of BMI and HOMA to changes in the response variable. Additionally, a multiple logistic regression model assessed the risk of high HOMA associated with high nesfatin-1 levels while controlling for age, gender, BMI, and duration of DM.

3 RESULTS

3.1 Serum Nesfatin-1 in T2DM Groups and Healthy Subjects

The level of serum nesfatin-1 decrease significantly in diabetic patients (55.12 ± 7.1 ng/ml)) when compared with control group (79.03 ± 11.10) p-value >0.05, Figure 1.

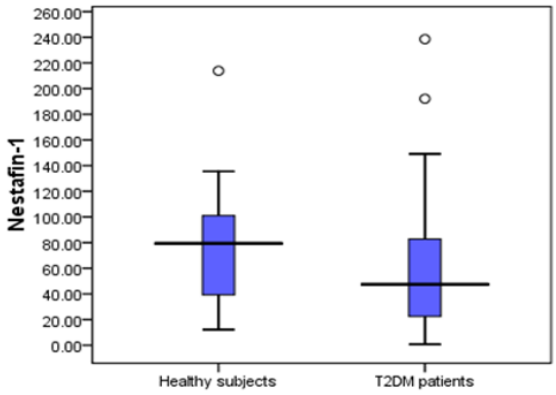


Figure 1: Nesfatin-1serum level in T2DM patients and controls.

3.2 Analysis of the Receiver Operator Curve (ROC)

The ROC analysis was used to distinguish between T2DM patients and controls using the following characteristics. Such an analysis enables the parameters to be organized according to the ROC region that they can occupy and whether or not this occupation is important. The ROC analysis revealed the descending order (FPG = 0.949; serum insulin = 0.693; serum nesfatin-1 = 0.869 of parameters that showed a significant variation (Table 1).

Table 1: Receiver operator curve (ROC) study in T2DM patients and controls for the analyzed parameters.

Parameter	ROC Area	P ≤
Glucose Levels in Fasting Plasma (mg/dL)	0.949	0.001
Serum nesfatin-1 (ng/ml)	0.869	0.01
Serum Insulin (µIU/ml)	0.693	0.01

N.S.: Not significant (P > 0.05).

3.3 Obesity (BMI) Impact on Serum Nesfatin-1

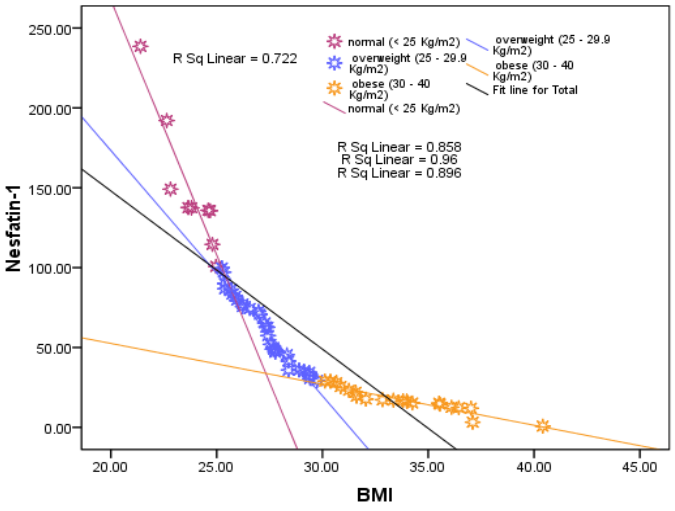
To examine the impact of obesity, as defined by BMI, on serum nesfatin-1 levels, we analysed the statistical differences in medians of nesfatin-1 among three obesity categories: normal (< 25 Kg/m²), overweight (25 - 29.9 Kg/m²), and obese (30 - 40 Kg/m²). These differences were assessed separately for patients with type 2 diabetes mellitus (T2DM) and control subjects. In T2DM patients, serum nesfatin-1 levels demonstrated a progressive decline across the obesity groups, with values of 148.86, 59.9864, and 16.7191 ng/ml for normal, overweight, and obese individuals, respectively. In control subjects, the corresponding values were 122.762, 77.4831, and 32.0860 ng/ml. The differences observed were statistically significant in T2DM patients (P ≤ 0.05) and controls (P ≤ 0.001, as shown in Table 2). Additionally, the correlation coefficients (r values of -0.346 and -0.72, respectively) were also significant (P ≤ 0.01 and P ≤ 0.001, respectively) for both groups, as illustrated in Figure 2.

3.4 Adjusted Effect of BMI and HOMA

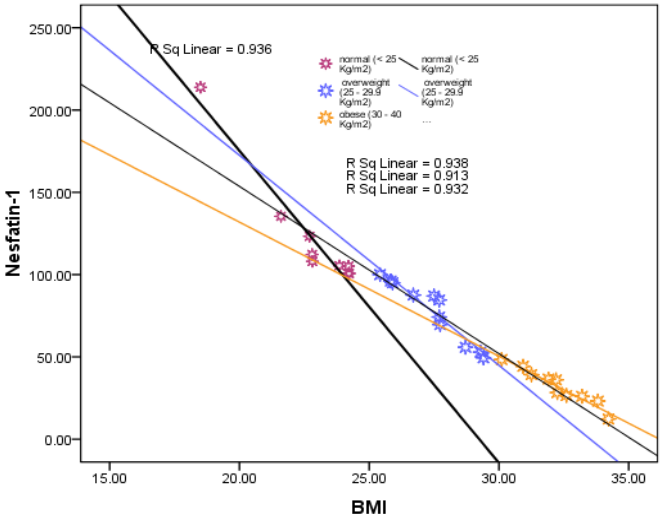
A multiple linear regression model was employed to study the net and independent effect of BMI and HOMA after adjustment for case-control differences on a set of selected outcomes (dependent) variables. The variables (nesfatin-1) were first log10-transformed before inclusion in the model (to normalize their distribution function). Accordingly, the model presented the standardized regression coefficient, which does not indicate the absolute change in the dependent (outcome) variable in response to the explanatory variables included in the model.

Table 2: Nesfatin-1 serum level in diabetic patients and controls defined as a result of obesity (body mass index).

Subjects	Obesity by BMI (kg/m^2)	N	Mean	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Patients	Normal (< 25 Kg/m2)	9	148.86	13.94677	116.9186	181.2412
	Overweight (25 - 29.9 Kg/m2)	32	59.9864	3.87584	52.0816	67.8913
	Obese (30 - 40 Kg/m2)	19	16.7191	1.65607	13.2398	20.1984
	Kruskal-Wallis Probability ≤ 0.05					
	r = -0.849; P ≤ 0.01					
Controls	Normal (< 25 Kg/m2)	9	122.762	35.99973	11.99991	95.0924
	Overweight (25 - 29.9 Kg/m2)	11	77.4831	18.26016	5.50564	65.2157
	Obese (30 - 40 Kg/m2)	10	32.0860	10.83410	3.42604	24.3358
	Kruskal-Wallis Probability ≤ 0.001					
	r = -0.96; P ≤ 0.001					



(a)



(b)

Figure 2: Correlation between serum nesfatin-1 levels and BMI in obese patients and healthy controls: a) Obese patients (n = 60); b) Healthy controls (n = 30).

Table 3: Multiple linear regression models with investigated variables (Log10) as the dependent (response) variable and age, gender, HOMA, BMI and case-control group membership as the independent variables.

Parameter-Log[10]	Standardized Partial Regression Coefficient (SPRC)			R^2 (Model)
	Being a Case with T2DM Compared to Controls	HOMA	BMI	
Serum nesfatin-1 (ng/ml)	-0.234	-0.16	-0.227*	0.285*

*Significant ($P \leq 0.05$) correlation.

Table 4: Multiple logistic regression models with the risk of having a high HOMA (highest quartile) among cases with T2DM after adjusting for age, gender, BMI and duration of T2DM and nesfatin-1.

The Highest Quartile of Parameter	Adjusted OR	Inverse OR	P ≤	P (Model) ≤	Overall Prediction Accuracy (%)
Serum Nesfatin-1(ng/ml)	33.3	0.03	0.05	0.01	74

OR: Odds ratio; P: Probability

Instead, its value points to the relative contribution of each independent variable to observed changes in the response variable. Each model compares the relative importance of BMI and HOMA (adjusted for each other) in deciding the magnitude of a specific dependent (response) variable. The analysis was presented as a standardized partial regression coefficient (SPRC). As shown in Table 3, being a person with diabetes is associated with a reduction in serum nesfatin-1 compared to controls (SPRC = -0.234). A change in BMI was more important in deciding serum nesfatin-1 than the HOMA index. Both BMI and HOMA had a negative association with serum nesfatin-1 (SPRC=-0.227 and -0.160, respectively). The regression model was statistically significant and explained 28.5% of the observed variation in the outcome variable (serum nesfatin-1).

3.4 The Risk of having High HOMA by Selected Parameters

The risk estimates (odds ratio; OR) in each model were adjusted for potential confounding variables, including age, gender, BMI, and duration of diabetes mellitus (DM). The logistic model demonstrated statistical significance, with prediction accuracy varying between 72% and 81%. Notably, the risk estimates were significant for high serum nesfatin-1 (OR = 33.30), indicating that elevated levels of serum nesfatin-1 significantly reduced the likelihood of having a high HOMA by 33.3 times when compared to individuals with average or low serum nesfatin-1 levels. See Table 4 for further details.

4 DISCUSSIONS

The present study found a statistically significant decrease in nesfatin-1 levels in patients with type 2 diabetes mellitus (T2DM) compared to control subjects. This finding aligns with the results of Liu et al. [14] and Li et al. [15], who also reported reduced nesfatin-1 levels in T2DM patients when compared to controls. In both the T2DM and control groups, serum nesfatin-1 levels exhibited an inverse correlation with body mass index (BMI). Previous research has reported low circulating levels of nesfatin-1 in individuals with obesity [14], [15]. study by Wang et al. [16] demonstrated that NUCB2, the precursor protein of nesfatin-1, has a polymorphism associated with metabolic syndrome and obesity. Specifically, the 1012C > G polymorphism in the NUCB2 gene has been linked to an increased risk of these conditions Additional studies looked at the relationship between anthropometric measurements of adiposity and DM risk. The results of Venkatrao et al.'s study [17], which showed that BMI and waist circumference were both valid markers of T2DM risk, are corroborated by our investigation. Furthermore, Grey et al. [18] found found being overweight and obese were important risk factors for type 2 diabetes and its effects in both men and women. Diabetes was more common in both males and females in the overweight category (BMI of 25–29.99).

The multiple linear regression analysis revealed an inverse correlation between BMI and HOMA, and serum nesfatin-1 levels; however, only the effect of BMI was statistically significant. Our results are consistent with those of Mohammad and Gallaly [19], and Matta et al.[20], who found a negative connection between serum nesfatin-1 level and HbA1c and fasting plasma glucose in diabetic individuals.

Strengths can be noted in our report. First, we matched the two groups based on age and BMI. Second, participants with T2DM were administered only one type of medication. However, this study also has a few limitations. The sample size was relatively small, and further rigorous follow-up studies on nesfatin-1 levels may provide better insights into the relationship between serum nesfatin-1 levels and the progression of T2DM.

5 CONCLUSIONS

This study demonstrated that serum nesfatin-1 levels are significantly lower in patients with type 2 diabetes mellitus (T2DM) compared to healthy controls. A strong negative correlation was observed between nesfatin-1 levels, body mass index (BMI), and insulin resistance (HOMA-IR), indicating its close association with metabolic disturbances and obesity.

The lowest nesfatin-1 levels were found in obese patients with T2DM, supporting its potential role as a marker of metabolic risk. Regression and ROC analyses further suggested that nesfatin-1 may have diagnostic value in assessing the risk and progression of T2DM, particularly in the presence of obesity and insulin resistance.

In conclusion, reduced nesfatin-1 levels are associated with an increased risk of type 2 diabetes and worsening metabolic status. These findings highlight the potential of nesfatin-1 as a promising biomarker for early diagnosis and monitoring of metabolic disorders in obese individuals with T2DM.

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