

Association of appetite hormone levels with HbA1c in obese type-2 diabetes mellitus patients

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38 Association of appetite hormone levels with HbA1c in obese type-2 diabetes mellitus patients



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ABSTRACT

Background: Nesfatin-1 and leptin are hormones that control appetite and affect glycemic control. Nesfatin-1 and leptin levels are affected by body mass index (BMI). Hemoglobin A1c (HbA1c) is an objective parameter to assess long-term glycemic control. This present research aims to know the relationship between levels of nesfatin-1 and leptin with HbA1c values as an indicator of long-term objective glycemic control in patients with T2DM with obesity. 24

Methods: Cross-sectional study on 54 obese T2DM subjects. Measurement of nesfatin-1 and leptin levels using the enzyme linked immunosorbent assay (ELISA) method. The value of HbA1c was measured by the fluorescent immunoassay (FIA) method. The correlation between nesfatin-1 and leptin with Hb1c was tested using the Spearman correlation analysis test ($p < 0.05$ was considered significant).

Results: The median value of nesfatin-1 levels was 1,824.0 (134.0-5,255.0) pg/mL, the median value of leptin levels was 14.0 (0.4 - 66.4) ng/mL, and the median value of HbA1c was 7.70 (4.9-15.0)%. There is no correlation between nesfatin-1 levels and HbA1c values ($p = 0.282$ and $r = 0.149$). There is no correlation between leptin levels and HbA1c values (p value = 0.895 and $r = 0.018$).

Conclusion: HbA1c levels are not associated with an increased appetite, as evidenced by no association between appetite biomarkers and glycemic control.

Keywords: HbA1c, Leptin, Nesfatin-1, Obesity, type 2 diabetes mellitus.

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INTRODUCTION

35 Diabetes mellitus (DM) is a chronic disease that occurs when the pancreas cannot produce insulin, or the body cannot use the insulin it produces effectively (known as insulin resistance / IR). Hyperglycemia is a common effect of uncontrolled diabetes and over time can cause serious damage to the body's systems, especially nerves and blood vessels. DM prevalence in Indonesia continues to increase.¹ Type 2 Diabetes mellitus (T2DM) is more common and mostly caused by being overweight and lacking physical activity.² Obesity is one of the main causes of death and is known as a major risk factor for non-communicable diseases, especially T2DM. Obese subjects had an increased amount of nonesterified fatty acids, glycerol, hormones, proinflammatory cytokines, and other substances involved in the formation of IR and development of T2DM.³ The prevalence of obesity varies

widely throughout the world, ages 18-60 years have the highest percentage of obese subjects compared to other age ranges.⁴

Obesity is strongly influenced by a person's intake and diet. Hunger is influenced by many factors such as levels of nesfatin-1 and leptin. Satiety and adiposity interact with other factors in the hypothalamus and elsewhere in the brain to control appetite and weight. Nesfatin-1 and leptin are parameters of concern for obesity and metabolic syndrome. There have been many studies on the relationship of obesity, nesfatin-1, and leptin separately.⁵

Nesfatin-1, first discovered in 2006, is a peptide involved in energy homeostasis by regulating appetite and drinking. Nesfatin-1 secretion increases when full and decrease during fasting.² It is secreted by peripheral tissues, the central nervous system (hypothalamic nucleus and brain stem) and the peripheral nervous system. Hunger decreases nesfatin-1 activity in

hypothalamic paraventricular neurons via the melanocortin pathway.⁶⁻⁹ An experimental study in obese mice with a leptin knockdown gene also reported that nesfatin-1 inhibits appetite.⁵ High nesfatin-1 levels in plasma are associated with obesity. This is supported by the Stengel cohort which showed the presence of genetic polymorphisms in NUCB2 (the gene that produces Nesfatin-1 protein) could cause obesity. The same study also showed that nesfatin-1 level responded to changes in adipose tissue mass.¹⁰ Nesfatin-1 plays an important role in the regulation of food intake in obese children and adolescents.¹¹

Leptin is a protein hormone that regulates the balance of food intake and energy expenditure through communication with the central nervous system. Leptin interacts with the mesolimbic dopamine system outside the hypothalamus which affects appetite and the nucleus tractus solitarius of the

brainstem to regulate satiety. Leptin is an important adipokine hormone released from adipose tissue, so its levels are higher in obese patients who have more fat cells.^{3,5} Leptin levels decrease during fasting or starvation and increase after eating. Leptin deficiency or resistance can result in severe obesity followed by insulin resistance (IR), conversely, obesity can also cause leptin resistance. Leptin resistance occurs because leptin secretion continues to increase in obese individuals so that it reaches saturation levels at its receptors.^{4,12} Sadia's research shows a strong relationship between leptin levels and obesity regardless of the presence of DM.¹³

Hemoglobin A1c (HbA1c) value has long been used as glycemic control tools in T2DM, which reflects treatment compliance and glycemic control according to the age of hemoglobin (about 120 days). A decrease in HbA1c values is associated with a decrease in the incidence of diabetes complications.¹⁴ The relationship between obesity and glycemic control shows varying results in various studies. Rakesh et al. showed a significant relationship between BMI and HbA1c values, while Emelia et al., found no relationship between obesity and glycemic control status as measured by HbA1c.^{15,16}

Based on the description above, nesfatin-1 and leptin affect obesity so that they can affect glycemic control compliance in T2DM patients. Adiposity in obesity is also a factor that affects nesfatin-1 and leptin levels, so the researchers wanted to know the relationship between levels of nesfatin-1 and leptin with HbA1c values as an indicator of long-term objective glycemic control in patients with T2DM with obesity.

METHODS

Study design

This cross-sectional study was conducted in March – July 2021 at the Program Pengelolaan Penyakit Kronis (PROLANIS/ Chronic Disease Management Program) at Manyaran Health Center, Miroto Health Center, Shafira Pratama Clinic, and Prolanis Banyumanik in Semarang.

Sample Collection

The target population is obese T2DM patients. Research subjects were selected by non-probability consecutive sampling, gave written consent after a thorough explanation and their identities were kept confidential. Inclusion criteria were age 18-60 years old, body mass index ≥ 25 kg/m², hemoglobin level >11 g/dL, and normal body temperature (36.4-37.2 °C). Exclusion criteria were consumption of certain drugs (corticosteroids, aspirin, chronic opioids, alcohol and antiviral drugs), history of splenectomy, history of or currently suffering from liver, heart, or gastric cancer, and currently undergoing insulin therapy.

Data were collected from history taking (interviews using questionnaires to obtain data of age, gender, disease (T2DM) history, use of drugs, insulin therapy, and co-morbidities), physical examination to measure vital signs, and anthropometric examination (height and weight) to establish the state of obesity. Diagnosis of T2DM was made by a health center doctor. Obesity criteria were body mass index (BMI) >25.0 kg/m² based on criteria by the Indonesian Health Ministry (Direktorat Pencegahan dan Pengendalian Penyakit Tidak Menular/ Directorate of Prevention and Control of Non-Communicable Diseases).

Venous blood was collected to measure HbA1c, nesfatin-1, and leptin levels. Six mL of venous blood were taken using a vacutainer and divided into an ethylene diamine tetra-acetic acid (EDTA) tube and a tube without anticoagulant. The tubes were labeled and immediately sent to the laboratory. HbA1c was measured using EDTA blood immediately in the laboratory using the fluorescence immunoassay (FIA) method (normal range $<6.5\%$ (NGSP)). Serum was separated from the tube without anticoagulant after 30-45 minutes (centrifuged at 3,000 rpm for 15 minutes). The serum was stored in a refrigerator at -20°C (stable for up to 4 months) until the time of Nesfatin-1 and leptin examination. Examination of nesfatin-1 and leptin levels using serum samples by ELISA method according to the manufacturer's instructions. Nesfatin-1 and Leptin levels

were checked by following the ELISA procedure provided by the Elabscience nesfatin-1 and leptin ELISA kit in GAKI laboratory of Diponegoro University, Indonesia (normal range of Nesfatin-1 was 0.6-2.6 ug/L; the normal range of Leptin was 2.5-21.8 ng/mL).

Statistical Analysis

Data analysis includes descriptive analysis (distribution, frequency and mean) and hypothesis testing. Univariate analysis was carried out on each variable to determine the characteristics of the sample. Bivariate analysis was carried out to find the relationship between nesfatin-1 and leptin levels with HbA1c values using the Spearman test. The p value is considered significant if $p < 0.05$. The degree of relationship is said to be very weak ($r = 0.000-0.199$), weak ($r = 0.20-0.399$), moderate ($r = 0.4-0.599$), strong ($r = 0.6-0.799$), and very strong ($r = 0.80-1.00$).

RESULTS

A total of 59 obese T2DM subjects participated in this study. Subjects who met the inclusion criteria were only 54 subjects consisting of 12 males and 42 females. Research subject characteristics are listed in table 1.

Nesfatin-1 levels had a median (min-max) of 1,824.0 (134-5,255) pg/mL, while the median (min-max) of HbA1c was 7.70 (4.9-15.0) %. There was no correlation between nesfatin-1 levels and HbA1c ($r = 0.149$; and $p = 0.282$) (Table 2). Leptin levels had a median (min-max) of 114.0 (0.4 - 66.4) ng/mL, while the median (min-max) HbA1c value was 7.70 (4.9-15.0) %. There was no relationship between leptin levels and HbA1c values ($r = 0.018$; and $p = 0.895$) (Table 2).

DISCUSSION

This study showed that nesfatin-1 levels did not have a significant correlation with HbA1c. This study is not in line with Xu's study in 2021 which analyzed the relationship between nesfatin-1 levels and HbA1c in diabetics regardless of BMI status. The results of Xu's study showed a significant relationship between the value

Table 1. Research Subject Characteristics.

Characteristics (n=54)	Mean ± SD	Median (min-max)
Age (year)	52.02 ± 6.37	54 (30-60)
Weight (kg)	74.46 ± 12.58	70.95 (55.40-152.80)
Height (cm)	154.26 ± 8.02	153.25 (134-177)
Body mass index (Kg/m ²)	31.42 ± 6.91	29.44 (25.35-61.21)
Hemoglobin (g/dl)	13.27 ± 1.85	13.5 (11.0-16.80)
Leucocyte (x10 ³ /uL)	8.67 ± 2.59	8.53 (4.28-16.29)
Thrombocyte (x10 ³ /uL)	284.22 ± 74.40	274 (151-573)
Erythrocyte (x10 ⁶ /uL)	5.54 ± 5.47	4.85 (2.54-4.7)
Hematocrit (%)	39.11 ± 5.07	39.5 (15.2-47.6)
Fasting blood glucose (mg/dL)	178.32 ± 133.37	145.74 (71.21-981.19)
Urea (mg/dL)	25.11 ± 11.35	23.4 (10.9-92.0)
Creatinine (mg/dL)	1.04 ± 0.54	0.98 (0-4.6)
Total cholesterol (mg/dL)	212.78 ± 39.12	211.90 (134-308)
HDL (mg/dL)	48.96 ± 22.29	45 (23-162)
Triglyceride (mg/dL)	192.24 ± 112.95	166.5 (37-597)
HbA1c (%)	8.13 ± 2.23	7.70 (4.9-15.0)

Note: SD: standard deviation; min: minimal; max: maximal; n, number of samples.

Table 2. Correlation between nesfatin-1 and leptin levels with HbA1c.

Variable	Hba1c	
	r	p
Nesfatin-1 (pg/ml)	0.149	0.282
Leptin (ng/ml)	0.018	0.895

Note: Spearman's Test, * $p < 0.05$; r: correlation coefficient.

of HbA1c with levels of nesfatin-1 ($p = 0.035$) with a very weak coefficient of $r = 0.184$.¹⁷

The difference between Xu's study and this research is the strict exclusion of subjects. In Xu's study, 2-hour post-prandial blood sugar was examined in all study subjects and levels > 11.2 mmol/l who were excluded. All study subjects in Xu's study were not undergoing any treatment.¹⁷ Xu's findings showed that nesfatin-1 levels were significantly positively associated with insulin levels and the IR model. Some of the subjects in this study had very high blood sugar levels and could affect blood osmolality. This study also did not exclude subjects with suspicion of ketoacidosis and hyperglycemic hyperosmolar status as in the study of Xu.¹⁷

This study showed that there were a number of subjects with elevated levels of urea and creatinine which may indicate the presence of diabetic nephropathy. Korani concluded that plasma nesfatin-1 levels were significantly higher in patients with diabetic kidney disease, and that there was a positive correlation between plasma nesfatin-1 levels and the degree

of kidney disease. Plasma nesfatin-1 can be considered for prediction of the development of diabetic kidney disease in previously unaffected DM patients.¹⁸

A number of subjects in this study were found to have elevated cholesterol and triglyceride levels which may affect nesfatin-1 levels. Rui Wu conducted an experimental study on mice by giving a high-fat diet to create a mouse model with increased levels of cholesterol and triglycerides in the blood. The findings of Rui Wu's study showed that nesfatin-1 levels were significantly associated with increased cholesterol and triglyceride levels.¹⁹

This study showed that leptin was not associated with HbA1c values. This study is in line with the case control study by Ramachandran which showed no significant correlation between leptin levels and HbA1c values with p value = 0.153.²⁰ Another study conducted by Maryam in female subjects with T2DM and BMI between 25-30 kg/m² showed results that were in line with this study where leptin levels were not associated with HbA1c values.²¹ Maryam also observed a negative relationship between leptin levels

and HbA1c in diabetic subjects, but it was not significant.

Based on the findings of this study and a review of the existing literatures, levels of nesfatin-1 and leptin fluctuate greatly and are influenced by many factors. Their levels can vary over days or shorter periods of time according to changes in the underlying factors. HbA1c is one parameter that reflects changes in glycemic control in the chronic period, so the relationship between nesfatin-1 and leptin may be difficult to identify with HbA1c values. Changes in nesfatin-1 and leptin levels may differ markedly from individual final HbA1c values over the course of the clinical course.

This study is the first study regarding the association between nesfatin-1 and leptin with HbA1c. Further research is needed to assess the relationship between nesfatin-1 and leptin in order to obtain stronger conclusions. Some of the limitations of this study that can be used for advanced research, among others: are gender classification as hormonal conditions can affect leptin levels, classification of oral antidiabetic drugs taken by patients, stricter exclusion criteria (blood sugar levels at the time of blood sampling, insulin levels, dehydration status, lipid levels, blood osmolality) as it can affect the results of the examination of nesfatin-1 levels and leptin levels. Besides, future researches need to evaluate other appetite markers with control glycemic marker (HbA1c) to provide exact appetite markers that are useful for monitoring and evaluation of T2DM patients.

CONCLUSION

HbA1c levels are not associated with an increased appetite, as evidenced by no association between appetite biomarkers and glycemic control.

AUTHOR CONTRIBUTION

EKSL and NSW were involved in supervising the research, analyzing data, and compiling the manuscript. SP was involved in research planning, measurements, data analysis and compiling the manuscript. MH was involved in analyzing data and reviewing and editing manuscript drafts. All authors discussed the results and commented.

ETHICAL CLEARANCE

The ethical permit was obtained from the Ethics Commission of Diponegoro University Semarang with the number 293/EC/KEPK/FK-UNDIP/VIII/2021.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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PAGE 2

PAGE 3

PAGE 4
