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ORIGINAL ARTICLE

Maternal Neudesin Levels in Pregnant Women with Gestational Diabetes Mellitus

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SUMMARY

Background: **Our aim was to investigate the changes in neudesin levels in pregnant women with GDM and the relationship between neudesin and metabolic parameters.**
Methods: **Forty pregnant women diagnosed with GDM and forty age- and gestational week-matched control subjects were included in the study. Demographic data were obtained from records. Maternal lipid profiles, glucose levels, fasting insulin, HbA1C, and HOMA-IR results were compared between the groups. Correlation tests were performed to evaluate the relationship between neudesin and clinical and laboratory diagnostic parameters. $p < 0.05$ were interpreted as statistically significant.**
Results: **The human serum neudesin levels were significantly lower in the GDM group compared with the controls. The correlation tests showed statistically negative and weak correlations between the neudesin levels and the maternal age, 50 g OGCT, 100 g OGTT 3 hours, and HbA1C. The optimum neudesin cutoff value for a diagnosis of GDM disease is 6.94 ng/dL, with a sensitivity of 65.9% and a specificity of 63.2%.**
Conclusions: **This study has shown that lower neudesin levels may occur as a reflection of changes in glucose metabolism during intrauterine life.**
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KEYWORDS

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INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as a glucose intolerance of varying degrees that begins during pregnancy or is first diagnosed during pregnancy [1]. GDM is a specific form of insulin resistance that occurs during pregnancy despite beta cell hyperplasia. This situation occurs when pregnancy-specific pancreatic beta cell hyperplasia cannot overcome the diabetogenic hormonal environment [1,2]. Many molecules such as adipokines, inflammatory markers, cytokines, and oxidative stress markers have been the main object of studies to clarify the pathogenesis of GDM [1-4]. However, the

etiopathogenesis is still not clear. In addition to genetic factors, obesity, excessive caloric intake, and inflammatory processes leading to insulin resistance and beta cell dysfunction, triggered by placental hormones and cytokines during pregnancy, are thought to play an important role in the etiopathogenesis of the disease [1-5]. With increasing obesity rates worldwide, GDM has become one of the most common pregnancy complications and remains significantly associated with an increased maternal-fetal morbidity and mortality [6-8]. Neudesin is a neuropeptide that belongs to the membrane-associated progesterone receptor (MAPR) family and is expressed in several tissues including the brain, the heart, and the kidney [9,10]. The MAPR family includes several components that are strongly associated with lipid and energy metabolism and the neuroprotection in the brain [11,12]. While white adipose tissue stores excess energy in the form of triglycerides, brown adipose tissue protects against obesity by releasing energy in the form of heat [9,10]. The sympathetic nervous system (SNS) plays an important role in the energy homeostasis. Studies in rodent models showed that neudesin protects knockout mice from insulin resistance [13]. Neudesin-blocked mice had increased energy expenditure with an increased sympathetic activity. This finding had paved the way for the study of the relationship between neudesin and energy metabolism [13]. Because this relationship became apparent, neudesin became an important subject of studies on obesity, insulin resistance, and diabetes mellitus.

Considering the relationship between neudesin and lipid and energy metabolism and the interaction with insulin resistance, we aimed to investigate the maternal neudesin levels in pregnant women with GDM and the relationship between neudesin levels, maternal blood lipid levels, and glucose metabolism parameters.

MATERIALS AND METHODS

A total of 80 pregnant women, 40 pregnant women diagnosed with GDM and 40 healthy cases matched in terms of gestational age and gestational week, were enrolled in our study. All cases were between 24 and 28 weeks of gestation. Cases with concomitant systemic diseases, gestational hypertension, gestational diabetes, multiple pregnancy, congenital anatomic and genetic anomalies, or with additional pregnancy complications, such as preterm delivery, chorioamnionitis, or intrauterine growth retardation, were excluded from the study. The diagnosis of GDM was made with a 2-step screening and diagnostic test. The diagnostic criteria were based on the guideline ACOG GDM. According to ACOG, in the first stage, a glucose value of more than 140 in the first hour after ingestion of 50 grams of oral glucose was considered a positive 50-gram OGCT. In the 2nd stage, we then performed a 100-gram OGTT for these cases and diagnosed GDM if the glucose values were as follows (diagnostic if 2 or more criteria were met). Fasting ≥ 95 mg/dL, 1 hour ≥ 180 mg/dL, 2 hours ≥ 155 mg/dL), 3 hours ≥ 140 mg/dL.

Demographic data were taken from the records. The venous blood samples were taken from the antecubital vein to analyze the neudesin level and other biochemical parameters after GDM diagnosis. Insulin resistance was measured using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). Fasting plasma insulin level and hemoglobin A1C level were also recorded for each subject. The blood samples were centrifuged at 1,000 g at 2 - 8°C for 15 minutes within 30 minutes of collection. The centrifuged samples were then cleared of plasma and stored at -80°C. After obtaining enough samples, the Human NENF (Neudesin) ELISA kits (Private Cagdas Laboratory) were analyzed at the Farmasina Istanbul Laboratories, according to the manufacturer's instructions.

After the approval of the study design by the Ethics Committee, the prospective case-control study was performed in the Department of Obstetrics and Gynecology, Ankara City Hospital, Ankara, Turkey. It lasted between March and August, 2022, and informed consent was taken from all the participants before the data were collected and retrieved. The study was initiated and conducted in accordance with the tenets of the Declaration of Helsinki.

Statistical Package for Social Sciences Version 24.0 software (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA) was used for the statistical analysis. The distribution of variables was checked in each group using the Kolmogorov-Smirnov method. We presented the distributions with mean (standard deviation) and median (min - max). The independent-samples *t*-test and the Mann-Whitney U test were used for parametric and nonparametric variables. Spearman's correlation test was used to test the correlation between neudesin and other variables for each group. Receiver Operating Characteristic Analysis (ROC) was performed to determine the predictive value of neudesin for GDM. A p-value of < 0.05 was considered statistically significant.

RESULTS

Clinical, demographic, and biochemical results are shown in Table 1. There were no statistical differences between the groups in maternal age, gravidity, parity, and gestational age at the time of blood collection (p: 0.057, p: 0.184, p: 0.068, p: 0.736, respectively)**.** The GDM group had higher values of BMI, 50 g OGCT, fasting insulin, HbA1C, and HOMA-IR compared to the control group ($p < 0.001$, p: 0.001, p: 0.008, $p < 0.001$, p: 0.009, respectively). There was no significant difference in fasting glucose and lipid profiles such as total cholesterol, HDL, LDL, and triglycerides (p: 0.281, p: 0.187, p: 0.172, p: 0.576, p: 0.623, respectively)**.** The serum neudesin levels were significantly lower in the GDM group than in the control group (p: 0.016)**.** The

Table 1. Comparison of demographic, biochemical parameters, and neudesin levels between the groups.

	Study Group (GDM) n = 40	Control Group n = 40	p-value
Maternal age (years)	30.83 ± 5.00	28.58 ± 5.36	0.057
Gravidity (number)	3 [1 - 7]	3 [1 - 5]	0.184
Parity (number)	1 [0 - 3]	0 [0 - 4]	0.068
BMI (kg/m^2)	31.21 ± 5.20	26.92 ± 4.56	< 0.001 *
Gestational age during blood sampling (week)	26.2 ± 1.5	26.1 ± 1.5	0.736
Total cholesterol (mg/dL)	236 ± 47	219 ± 39	0.187
HDL (mg/dL)	79 ± 33	69 ± 22	0.172
LDL (mg/dL)	115 ± 44.31	110.16 ± 31.37	0.576
Triglyceride (mg/dL)	214.27 ± 58.36	207.42 ± 65.09	0.623
Fasting glucose (mg/dL)	81.98 ± 20.01	78.29 ± 8.07	0.281
Fasting insulin (µIU/mL)	11.8 ± 609	10.6 ± 9.6	0.008 *
Homa-IR	3.5 ± 3.7	2.3 ± 3.6	0.009 *
HBA1C (%)	5.72 ± 0.54	4.45 ± 0.60	< 0.001 *
Human neudesin(ng/dL)	7.1 ± 5.2	10.5 ± 8	0.016 *
50 g OGCT, 1 hour (mg/dL)	162.2 ± 28	162.2 ± 28	0.001 *
100 g OGTT, 0 hour (mg/dL)	89.5 ± 9.9		
100 g OGTT, 1 hour (mg/dL)	190 ± 22.8		
100 g OGTT, 2 hours (mg/dL)]	162.2 ± 28		
100 g OGTT, 3 hours (mg/dL)	122 ± 26.4		

Data are expressed as mean (standard deviation), median (minimum-maximum).
*** p-value < 0.05 indicates a significant difference.**
OGCT - Oral Glucose Challenge Test, OGTT - Oral Glucose Tolerance Test, BMI - Body mass index.

Table 2. Correlation analysis of different parameters with the neudesin levels in the study group.

Variables	Neudesin	
	r **	p
Maternal age	-0.468	0.002 *
BMI (kg/m^2)	-0.004	0.970
50 g OGCT, 1 hour (mg/dL)	-0.216	0.048 *
100 g OGTT, 0 hour (mg/dL)	-0.065	0.569
100 g OGTT, 1 hours (mg/dL)	-0.178	0.117
100 g OGTT, 2 hours (mg/dL)	-0.142	0.211
100 g OGTT, 3 hours (mg/dL)	-0.240	0.033 *
Total cholesterol (mg/dL)	-0,106	0.352
HDL	-0.197	0.081
LDL	0.090	0.428
Triglyceride (mg/dL)	-0.153	0.179
Fasting insulin (µIU/mL)	-0.094	0.409
Homa-IR	-0.179	0.114
HBA1C (%)	-0.242	0.031 *

**** Spearman's and Pearson's correlation coefficient.**
*** p-value < 0.05 indicates a significant difference.**
OGCT - Oral Glucose Challenge Test, OGTT - Oral Glucose Tolerance Test, BMI - Body mass index.

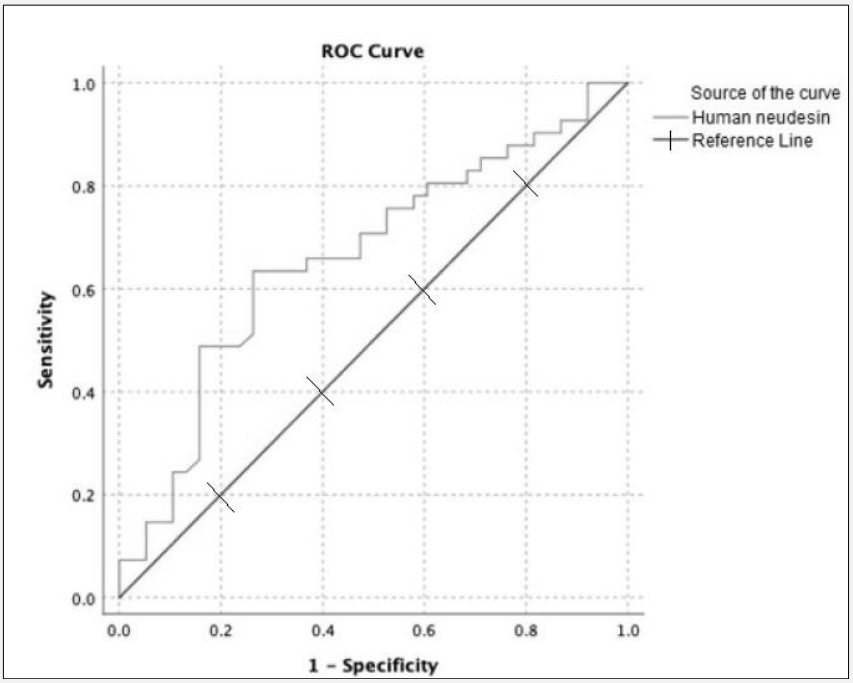

Figure 1. ROC curve for neudesin level in predicting GDM disease.

levels of 100 g OGTT 0 hour (mg/dL), 100 g OGTT 1 hour (mg/dL), 100 g OGTT 2 hours (mg/dL), and 100 g OGTT 3 hours (mg/dL) in the study group were 89.5 ± 9.9, 190 ± 22.8, 162.2 ± 28, and 122 ± 26.4, respectively.

In the study group, Spearman's and Pearson's correlation tests showed statistically negative and weak correlations between neudesin and maternal age, 50 g OGCT, 100 g OGTT 3 hours, and HbA1C (Table 2) (p: 0.002, p: 0.048, p: 0.033, and p: 0.031, respectively). There were no statistically significant correlations between neudesin and BMI, fasting insulin, HOMA-IR, 100 g OGTT 0 hour, 100 g OGTT 1 hour, 100 g OGTT 2 hours, as shown in Table 2. The optimal Neudesin cutoff value for the diagnosis of GDM disease was determined to be 6.94 ng/dL in the ROC curve analyzes, with a sensitivity of 65.9% and a specificity of 63.2% (Figure 1).

DISCUSSION

To our knowledge, this is the first study investigating the relationship between neudesin, GDM, and the lipid profile during pregnancy. In this prospective case-control study, we found lower neudesin levels in pregnant women diagnosed with GDM compared with the control group. Spearman and Pearson correlation tests showed statistically negative and weak correlations between the neudesin levels and the maternal age, 50 g OGCT, 100 g OGTT 3 hours, and HbA1C. However, there were no statistical correlations between Neudesin and BMI, fasting insulin, HOMA-IR, 100 g OGTT 0 hour, 100 g OGTT 1 hour, 100 g OGTT 2 hours. We also showed that the optimal neudesin cutoff value for the diagnosis of the GDM disease is 6.94 ng/dL, with a sensitivity of 65.9% and a specificity of 63.2%.

Neudesin is a multitasking molecule that may play a role in cell differentiation, proliferation, energy and carbohydrate metabolism, obesity related metabolic diseases, and tumorogenesis [9-10,14-16]. The peripheral and central activity potential of neudesin may be quite different. It was found that after the administration of recombinant neudesin into the cerebral ventricle, there was a decrease in food intake and body weight and an increase in pro-opiomelanocortin and melanocortin 4 receptor expression in the hypothalamus [17]. However, in heavily fed obese mice, the effects of neudesin on food intake were abolished, and these mice appeared to be resistant to obesity. These results suggest that hypothalamic neudesin is an important modulator of food intake [10,11,17].

Neudesin, which is known to be expressed in white adipocytes, has been shown to inhibit adipogenesis in cul-

tured preadipocytes [1]. This suggests that peripheral neudesin may have different effects than centrally released neudesin, or that the peripheral effects of neudesin are different. Studies have shown that peripheral neudesin blocks lipolysis in white adipose tissue and fatty acid oxidation and heat production in brown adipose tissue by inhibiting sympathetic nerve activity [17].

Obesity, which develops due to an excessive development of white fat, is an important risk factor for various metabolic diseases such as type II diabetes, hypertension, and atherosclerosis [18-21]. The relationship between insulin and various metabolic pathways, such as WNT and MAPK, has been studied previously [22]. These signaling pathways and their effects, especially at the adipocyte level, are closely related to activation by phosphorylation of proteins such as Akt and ERK 1/2, which are also necessary for the action of insulin [23]. ERK activation and phosphorylation have been identified as triggers for beta cell proliferation in animal models of obesity [22,23]. Although the mechanisms are so complex, it cannot be assumed that each molecule has the same habitual effect in each organ system. Considering the release of neudesin in white adipose tissue, its modulatory effect on food intake, and its role in adipogenesis, the relationship between neudesin and glucose metabolism has been addressed as an intriguing issue in current studies.

The number of studies investigating the neudesin levels in the pregnant population and in patients diagnosed with GDM is quite limited [24]. The only study examining neudesin in GDM was conducted by Eren et al. In this study, 24 pregnant women diagnosed with GDM (cases regulated by diet and exercise only) and 23 healthy pregnant women were compared in terms of maternal serum and umbilical cord neudesin levels [24]. The study reported that the maternal serum neudesin levels were significantly higher in the GDM group. In addition, it showed that there is a positive correlation between HOMAIR and the insulin levels that are related to the glucose metabolism and the maternal neudesin levels. In contrast to this study, we found that the maternal neudesin levels were lower in the GDM cases than in the control group. In contrast to the study by Eren et al., our study population included a larger number of cases (n: 80). All of our 40 GDM cases received insulin therapy in addition to exercise and a specific diet. In our study, in addition to fasting insulin and HOMA-IR, we also investigated whether there was a correlation between maternal blood lipid parameters, 50 g OGCT, 100 g 3-hours OGTT glucose parameters, and neudesin. We were unable to demonstrate the positive relationship that Eren et al. found between neudesin, HOMAIR, and fasting insulin. However, we found negative and weak correlations between the neudesin levels and maternal age, 50 g OGCT, 100 g OGTT 3-hours, and HbA1C. In our study, although the BMI was significantly higher in the GDM group, we did not find any correlation between the BMI and the neudesin levels. Insulin levels were higher in the GDM group. Considering the similar effects of neudesin and insulin on adipogenesis and the different results for caloric intake and central neudesin effects, it is reasonable to assume that the many different mechanisms may lead to the different results.

Our study has some limitations. The groups in our study were not matched for BMI. However, there was no statistical association between the BMI and the neudesin in this current study. Subgrouping by BMI levels will contribute to our understanding of whether obesity, which plays a role in the development of diabetes, or diabetes itself is associated with a decreasing neudesin. A prospective study with more participants will show the importance of neudesin in GDM cases more clearly.

In conclusion, our study is, to the best of our knowledge, the second study 1) examining the relationship between neudesin, gestational diabetes, and the lipid profiles and 2) emphasizing the importance of the relationship between the three and the glucose metabolism. We found in our study that there is a negative correlation between the glucose levels and the neudesin levels, which play a modulatory role in many mechanisms with its peripheral and central effects. Our results need to be supported by large series, prospective, randomized and subgroup studies.

Declaration of Interest:

None.

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