Research Article

Low apelin levels are associated with a marked increase in risk of gestational diabetes mellitus development

Düşük apelin seviyeleri, gestasyonel diabetes mellitus gelişme riskinde artış ile ilişkilidir

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ABSTRACT

Background: Apelin is an adipokine which plays a role in the regulation of glucose homeostasis. Relationships between serum apelin concentrations and dysmetabolic conditions are still controversial. The aim of this study was to investigate the association of serum apelin levels with gestational diabetes mellitus (GDM).

Material and Methods: This study was designed as a cross-sectional research that consecutively recruited subjects with GDM (n=38), without GDM pregnant (n=41), and non-pregnant healthy women (n=39). Fasting blood glucose (FBG), serum apelin, insulin and lipids were measured. BMI and HOMA-IR were calculated for all subjects. Logistic regression analysis was performed to determine predictors of GDM development.

Results: Serum apelin levels (GDM = 1.99 ± 1.36 mg/ml, non-GDM pregnant = 2.95 ± 1.36 mg/ml, non-pregnant women = 2.62 ± 1.67 mg/ml) were significantly lower ($p = 0.017$), HOMA-IR ($p = 0.024$) and BMI ($p < 0.001$) were significantly higher in the GDM group compared with both the non-GDM and the nonpregnant women. Serum apelin levels were found to be negatively correlated with FBG ($r = -0.236$, $p = 0.010$), OGTT 1 h glucose ($r = -0.346$, $p = 0.002$) & 2 h glucose ($r = -0.248$, $p = 0.028$), HbA1c ($r = -0.209$, $p = 0.023$), HOMA-IR ($r= -0.360$, $p < 0.001$) and BMI ($r = -0.299$, $p = 0.001$).

Conclusions: Serum apelin levels were significantly lower in the GDM group as compared with both the non-GDM pregnant and the non-pregnant healthy women. Low apelin levels appear to be an independent predictor of GDM development.

Keywords: GDM, Apelin, HOMA-IR, HbA1c

ÖZ

Amaç: Apelin, glikoz homeostazının düzenlenmesinde rol oynayan bir adipokindir. Serum apelin konsantrasyonları ile dismetabolik durumlar arasındaki ilişkiler hala tartışmalıdır. Bu çalışmanın amacı serum apelin düzeylerinin gestasyonel diabetes mellitus (GDM) ile ilişkisini araştırmaktır.

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Gereç ve Yöntem: Bu çalışma, ardışık olarak GDM'li (n=38), GDM olmayan gebe (n=41) ve gebe olmayan sağlıklı kadınların (n=39) alındığı kesitsel bir araştırma olarak tasarlanmıştır. Açlık kan şekeri (AKŞ), serum apelin, insülin ve lipidler ölçüldü. Tüm denekler için BMI ve HOMA-IR hesaplandı. GDM gelişimini öngördürücüleri belirlemek için lojistik regresyon analizi yapıldı.

Bulgular: Serum apelin seviyeleri (GDM = 1,99 ± 1,36 mg/ml, GDM olmayan gebe = 2,95 ± 1,36 mg/ml, gebe olmayan kadın = 2,62 ± 1,67 mg/ml) anlamlı olarak daha düşüktü (p = 0,017), HOMA-IR (p = 0,024) ve BMI (p < 0,001) GDM grubunda anlamlı olarak yüksekti. -GDM ve gebe olmayan kadınlar. Serum apelin düzeylerinin AKŞ (r = -0,236, p = 0,010), OGTT 1 saatlik glukoz (r = -0,346, p = 0,002) ve 2 saatlik glukoz (r = -0,248, p = 0,028), HbA1c (r = -0,209, p = 0,023), HOMA-IR (r= -0,36) ile negatif korelasyon gösterdiği bulundu. 0, p < 0,001) ve BMI (r = -0,299, p = 0,001).

Sonuç: Serum apelin düzeyleri GDM grubunda hem GDM olmayan gebelere hem de gebe olmayan sağlıklı kadınlara göre anlamlı derecede düşüktü. Düşük apelin seviyeleri, GDM gelişiminin bağımsız bir göstergesi gibi görünmektedir.

Anahtar Kelimeler: Apelin, gestasyonel diabetes mellitus, HOMA-IR

INTRODUCTION

Glucose intolerance that develops during pregnancy is called as gestational diabetes mellitus (GDM) (1). Parallel to the increase in the prevalence of diabetes all over the world, the incidence of GDM is also increasing due to the increase in maternal age and obesity. Although there are different results between countries, the prevalence of GDM is estimated between 1.7-11.6% in developed economies; the frequency is increasing in young and obese women (2). More than 200,000 pregnancies per year are complicated by GDM (1).

Pregnancy progresses with the development of the fetoplacental unit and a decrease in insulin sensitivity physiologically. The main purpose of these changes is to meet the nutrition and energy needs of the fetus, which is in continuous development. In response to the development of insulin resistance in target organs during pregnancy, insufficient insulin secretion of the pancreas causes hyperglycemia (3). GDM is the cause of significant complications in both the mother and the baby. While macrosomia, hypoglycemia, hyperbilirubinemia, congenital anomalies and respiratory distress syndrome are adverse neonatal outcomes, hypertension and preeclampsia are some of the possible maternal complications. Women with GDM and their babies are at increased risk of developing diabetes and metabolic syndrome later in life (4).

Recent developments have made it possible to examine the pathophysiology of insulin resistance at the molecular level, aiming to reveal the factors that cause insulin resistance and to develop new treatment and diagnostic strategies targeting these factors. In cell culture studies conducted in recent years, it has been determined that apelin peptide, an adipocytokine, plays an important role in the development of insulin resistance. Apelin receptors (APJ) are expressed in muscle, liver and adipose tissue, which are insulin sensitive tissues (5). It was observed that glucose uptake was increased in adipocytes after apelin injection into adipocytes that had insulin resistance with TNF alpha in vitro. It has been determined that apelin provides this effect by promoting GLUT4 translocation from the cytoplasm

to the plasma membrane via AMP-activated protein kinase (AMPK) and endothelial nitric oxide synthase (eNOS). Again, apelin inhibits triglyceride hydrolysis by inhibiting hormone sensitive lipase in adipose tissue, thus preventing free fatty acid release into the systemic circulation (6). Apelin increases the expression of peroxisome proliferator activated receptor γ coactivator 1α (PGC1α) with AMPK activation in muscle tissue. In this way, glucose uptake, beta oxidation, nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (TFAM) levels increase in muscle tissue. NRF-1 and TFAM increase mitochondrial biogenesis and oxidative phosphorylation capacity (7-8).

It was determined that oxidative phosphorylation capacity and fat oxidation in muscle cell mitochondria increased, white adipose tissue mass decreased, thermogenesis increased in brown adipose tissue, and as a result, insulin sensitivity improved in obese and insulin resistant mice with apelin administration (8-9). The fact that apelin increases glucose utilization in insulin-sensitive tissues in mice suggests that it may be a promising target molecule in the management of insulin resistance (5).

As it is known, the development of insulin resistance during pregnancy is to ensure optimal fetal nutrition. Accordingly, the effect of apelin on pregnancy metabolism is not fully known. In the study by Telejko et al. apelin and APJ (endogenous ligand of the G protein-coupled receptor of Apelin) mRNA levels investigated in serum, subcutaneous adipose tissue, visceral adipose tissue and placenta tissue of pregnant women with normal glucose tolerance and pregnant women with GDM were examined, and the relationship of apelin with the GDM process was evaluated.

Although a statistically significant relationship between apelin and apelin receptor expression with metabolic parameters was not detected, it was found that apelin mRNA expression in placental tissue was ten times higher than in adipose tissue. This result indicates that apelin plays an important role in the regulation of placental vascularization and blood flow, which is necessary for a normal fetal development (10-11).

Recognition and targeting of the factors that cause insulin resistance not only enables early diagnosis and treatment of GDM, but also creates a new threshold in the treatment of diabetes, metabolic syndrome and its complications. The role of apelin in the development of insulin resistance, which plays a potential role in the pathophysiology of GDM, was investigated in this study.

MATERIALS AND METHODS

Our study was designed as a case-controlled cross-sectional study. Ethics committee approval was obtained for our study with the decision of Dokuz Eylül University Non-Interventional Research Ethics Committee dated 10.05.2012 and numbered 2012/17-23. Our research was carried out between 01st September 2012 and 31st March 2013.

Research group

The research group consisted of three different groups of 40 people, each being pregnant women diagnosed with GDM, healthy pregnant women with similar age and gestational weeks, and healthy non-pregnant women with similar demographic characteristics. The pregnant women included in the study were 24-30 weeks pregnant women who were followed up in the Dokuz Eylul University Hospital Gynecology and Obstetrics clinic and referred to the Hospital Endocrinology clinic for oral glucose tolerance test (OGTT) screening. Pregnant women who volunteered to participate in the study were divided into two groups as those with GDM and healthy pregnant women according to the OGTT result. The healthy non-pregnant women in the research group were also composed of healthy women in the similar age range who came to the Endocrinology clinic of University for routine control

and examination and were in compliance with the research protocol.

Written consent was obtained from the subjects who accepted to participate in the study, and the Declaration of Helsinki was complied with during the study.

Inclusion criteria;

- To be over 18 years old
- To be pregnant in 24-28. weeks
- Volunteer to participate in research

Exclusion criteria;

- Having a diagnosis of malignancy
- Having a systemic disease (liver or kidney failure, adrenal, thyroid and parathyroid disease, metabolic bone disease, type 1 and type 2 diabetes, malabsorption syndrome or connective tissue disease)
- Chronic alcohol abuse
- Having a twin pregnancy
- Diagnosed with pre-pregnancy diabetes
- Use of drugs that affect carbohydrate and lipid metabolism

Clinical Evaluation and Specimen Collection

Demographic data, anthropometric measurements, medical and reproductive histories of the research group were recorded. The blood pressure of the cases was measured manually with Erka brand blood pressure device after 15 minutes of rest. Body weights were measured with light clothing and shoes removed. Height measurements were made with a standard measuring tape. Body mass index was calculated as kg/m2. In the study, GDM screening and diagnosis were made according to International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria (12,13). OGTT was performed in the morning after fasting for at least eight hours with 75 grams of glucose. The patients were given water-sugar solution within 10 minutes. For blood glucose measurements, antecubital venous blood samples were taken at 0, 60, and 120 minutes during OGTT. The test was repeated the next day in patients who developed emesis after drinking water-sugar solution. For other parameters determined in the study (fasting insulin, fasting C-peptide, apelin, hs-CRP, TSH, PTH, creatinine) from the blood sample obtained from the study group for at least eight hours of fasting, for fasting blood glucose determination, GGT, triglyceride, HDL, LDL, total cholesterol sample was separated. Serum samples were separated from the blood samples taken from the subjects by cold centrifugation method (4000 rpm/min, 15 min). The serum samples obtained were stored at -80 degrees. Two cases in the GDM group and one case in the control female group were excluded from the study due to insufficient serum samples.

Laboratory measurements

Serum apelin (Raybiotech Inc., USA, intra-assay CV< 10%, inter-assay CV< 15%) levels of the research group were measured by enzyme-linked immunosorbent assay (ELISA) method. Fasting blood glucose, and serum levels of hs-CRP, creatinine, GGT, ALT, triglyceride, total cholesterol, HDL, sodium, potassium, calcium, phosphorus in our study were measured by spectrophotometric method using Abbott Diagnostics original kits and Abbott Architect C16000 autoanalyzer (Illinois, USA). Fasting insulin level, PTH, TSH, fT4, fT3 levels in Abbott Diagnostics original kits with Abbott Architect I2000 autoanalyzer (Illinois, USA), fasting C-peptide levels in Cobas e601 autoanalyzer with Roche Diagnostics original kits (Manheim, Germany) were measured by electrochemiluminescence immunoassay method. HbA1c was measured by high-performance liquid chromatography (HPLC) method on an Adams HA-8160 Arkray autoanalyzer (Longfield, England).

All parameters in our research were studied in the Central Laboratory of Dokuz Eylul University Medical Faculty Hospital, which has ISO15189 accreditation certificate. Our research was supported by the Dokuz Eylul University Scientific Research

Fund. (Project number: 2012.KB.SAG.094). Insulin sensitivity of the patients was calculated according to QUICKI (Quantitative insulin sensitivity check index), insulin resistance according to HOMA-IR (Homeostasis model assessment) and pancreatic insulin secretion according to HOMA-β (homeostasis model assessment-beta).

• QUICKI-IS: 1 / [(log (fasting insulin) (μU/mL) + log (fasting glukoz) (mg/dL) (14).

• HOMA-IR = fasting insulin (μU/ mL) x fasting glucose (mmol / L) / 22.5 (15).

• HOMA-β = fasting insulin (μU/ml)x20) / fasting glucose (mmol/l) (15).

Statistical analysis

Statistical analyzes were performed in the PASW Statistics 18 program. Descriptive statistics; percentage distributions, mean and standard deviation values are presented. One Way Anova test was performed to compare the independent variables of GDM, healthy pregnant and non-pregnant healthy women. If a difference was detected between the three groups, post-hoc analysis was performed with Bonferroni correction to understand which group caused the difference. Pearson's correlation analysis and the relationship between clinical and laboratory characteristics were examined.

In order to determine the relative risk, a logistic regression model was created between GDM and healthy pregnant women. Evaluation of the results: The $p< 0.05$ value was accepted as the significance value, taking into account the 5% margin of error in the 95% confidence interval.

RESULTS

The study included 38 pregnant women with GDM as the patient group, 41 healthy pregnant women and 39 healthy non-pregnant women as the control group. Demographic and anthropometric characteristics of the study cases are shown in Table 1.

Table 1. Demographic characteristics of the research group

Variables	GDM (n=38)	Control pregnant (n=41)	Control non-pregnant (n=39)	p
Age, years	32±5	28±4	26±3	<0.001
Education, years (>11 years)	%34.2	%34.1	%100	<0.001
DM in the mother	%39.5	%17.4	%10.3	<0.001
BMI (prior to gestation), kg/m^2	27±5.4	23±3.9	20.1±2.1	<0.001
Blood pressure, mm Hg				
Systolic	110±10	103±11	100±10	<0.001
Diastolic	68±8	62±9	66±6	0.011
Pregnancy-related issues				
Gestational week	27±2	26±2	N/A	0.281
Gravida	2.2	2.0	N/A	0.346
Parida	0.9	0.8	N/A	0.627
History of GDM	%13.2	%2.4	N/A	0.098
Weight of the baby, gr	3147±559	3254±420	N/A	0.35

BMI: body-mass index; **DM:** diabetes mellitus**; GDM:** gestational diabetes mellitus; **N/A:** not applicable

The levels of HbA1c ($p<0.001$), serum fasting blood glucose ($p<0.001$), serum fasting C-peptide ($p=0.002$), HOMA insulin resistance index ($p=0.024$), serum triglyceride ($p<0.001$), serum hs-CRP ($p<0.001$) and serum GGT ($p=0.007$) of pregnant women with gestational diabetes were found to be statistically significantly higher than the control group.

Although the serum fasting insulin level of pregnant women with gestational diabetes was found to be higher than the control group, this was not significant ($p=0.786$). Serum apelin levels ($p=0.017$),

QUICKI insulin sensitivity (p=0.002) and serum creatinine levels (p<0.001) of the pregnant women with gestational diabetes were found to be statistically significantly lower than the control group. The laboratory characteristics of the research groups are shown in Table 2.

Table 2. Laboratory results of the research group (Values are shown as X±Standard deviation)

Variables	GDM (n=38)	Control pregnant (n=41)	Control non-pregnant (n=39)	p
HbA1c, %	5.4±0.5	4.9±0.3	5.1±0.2	<0.001
Fasting glucose, g/dL	87±20	74±7	88±11	<0.001
Insulin, µIU/mL	9.7±5.3	8.4±8.8	8.2±14.4	0.786
Fasting C-peptide, ng/mL	2.6±1.1	2.2±1.4	1.7±0.5	0.002
HOMA-IR	2,1±1,62	1,56±1,75	1,3±0,54	0.024
HOMA-β	40.6±19.1	41.4±41.4	24.4±9.1	0.012
Quicki	0.350±0.033	0.370±0.035	0.370±0.027	0.002
Apelin, µg/mL	1.99±1.36	2.95±1.36	2.62±1.67	0.017
Triglyceride, mg/dL	219±81	179±103	66±20	<0.001
hs-CRP, mg/L	7±6	6±6	1±1	<0.001
GGT, U/L	13±9	9±4	11±5	0.007
Creatinine (serum), mg/dL	0.57±0.07	0.59±0.09	0.68±0.08	<0.001
PTH (pg/mL)	29.6±13.6	29.9±20.3	37.6±17.8	0.078
TSH, µIU/mL	1.45±0.89	1.49±0.77	1.50±0.78	0.965

BMI: body-mass index; **DM**: diabetes mellitus; **GDM**: gestational diabetes mellitus

The pearson correlation analysis of serum apelin levels with other clinical and laboratory data were examined. A significant and positive correlation was found between serum apelin levels and QUICKI insulin sensitivity (r = 0.42, p < 0.001). A negative correlation was found between serum apelin levels and HOMA insulin resistance index (r = -0.36, p < 0.001), body-mass index (r = -0.29, p < 0.001), fasting serum glucose levels (r = -0.23, p = 0.01), fasting C-peptide levels (r = -0.372, p = 0.001) and HbA1c levels (r = -0.20, p = 0.023). There was no negative or positive correlation between serum apelin levels and age, gestational week, fasting insulin levels and serum hs-CRP. There was a strong positive correlation between serum apelin levels and QUICKI insulin sensitivity (r=0.82, p<0.001) in the pregnant group with GDM compared to other case groups. A negative correlation was observed between serum apelin levels and HOMA insulin resistance index (r=-0.61, p<0.001) in the pregnant group with GDM.

Serum apelin levels, HOMA insulin resistance index, age, body-mass index, hs-CRP, and maternal diabetes were included in the logistic regression model. In the multivariate analysis (Table 3), older age (OR 1.20, 95% CI, p<0.001) was associated with a higher risk of GDM, while a higher serum apelin level was protective against GDM (OR 0.60, 95% CI, p = 0.040).

Table 3. Predictors of gestational diabetes mellitus in multivariate regression analysis

Variables	OR	95% CI	p
Age	**1.20**	**1.05-1.40**	**<0.001**
BMI	1.11	0.97 1.28	0.120
DM in the mother	2.87	0.81-10.19	0.101
Apelin	**0.60**	**0.30-1.00**	**0.040**
HOMA-IR	1.00	0.68-1.46	0.970
hs-CRP	1.02	0.94-1.11	0.580

BMI: Body-mass index; **DM:** diabetes mellitus; **HOMA-IR:** Homeostasis model assessment of insulin resistance; **hs-CRP:** High sensitive C-reactive protein.

Women in all three groups were compared in terms of serum apelin level (Figure. 1-a). A statistically significant difference was found between them (p=0.017). In the post-hoc analysis, it was understood that this difference was due to the difference in serum apelin levels between pregnant women with GDM and healthy pregnant women. The serum apelin level was found to be low in women with GDM. HOMA insulin resistance index was found to be statistically significantly higher in pregnant women with gestational diabetes compared to the control women group (p=0.025) (Figure. 1-b). Triglyceride / HDL ratio (p<0.001) and C-peptide / FPG ratio (p=0.02), which are indirect indicators of insulin resistance, were found to be significantly higher in the pregnant group with GDM compared to the control female group (Figure. 1-c/d).

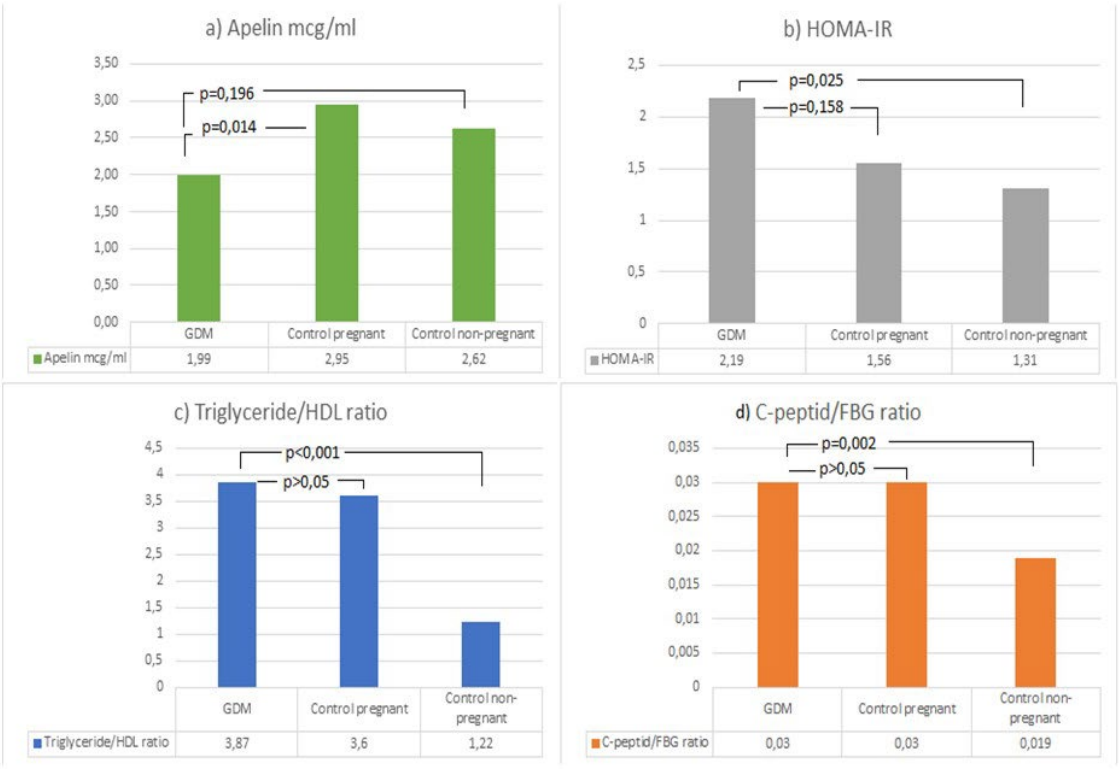

Figure 1. Serum apelin levels, HOMA-IR levels, triglyceride / HDL ratio, C-peptide/fasting blood glucose ratio in groups presented in figure 1.

DISCUSSION

The development of insulin resistance plays a fundamental role in the pathophysiology of GDM. Therefore, in our study, the effect of apelin, which is thought to be closely related to insulin resistance, on the development of GDM was examined. Apelin has been shown in cell culture and animal experiments to improve insulin sensitivity by increasing glucose utilization in insulin-sensitive tissues (5,8,9). In this study, serum apelin levels of pregnant women with GDM and control groups were determined and their relations with insulin resistance parameters were examined. Serum apelin level was found to be significantly lower in pregnant women with GDM compared to the control groups. While a significant and positive correlation was found between serum apelin level and QUICKI insulin sensitivity, a negative correlation was found between serum apelin level and HOMA insulin resistance index. In line with these analyzes, it was thought that there was a strong and important relationship between the development of GDM and serum apelin level.

Due to the role of apelin in placental blood flow and vasculogenesis, it is known that its production from the placenta increases in healthy pregnancy (12). According to the regression model in our study, it was determined that insufficient apelin production during pregnancy increased the risk of developing GDM (RR= 1.4, p=0.04). From this point of view, we think that insufficient apelin production or due to an increase in clereance during pregnancy is a factor that facilitates the development of GDM. There are few studies in the literature dealing with the effects of apelin on the physiology of pregnancy and the development of GDM (10- 11). In the research of Telejko et al., which includes the highest number of patients on this subject; 101 pregnant women with GDM and 101 pregnant women with normal glucose tolerance were included. There was no statistically significant difference between the two study groups in terms of serum apelin levels (10). In the same study, no correlation was observed between serum apelin level and insulin resistance indicators. In the study of Telejko et al., WHO criteria were used as the diagnostic criteria for GDM (75 g OGTT, serum fasting glucose was ≥ 100 mg/dl and ≥ 140mg/dl at 120. min) (11). This suggests that there may be pregnant women with GDM in the pregnant group, which was interpreted as normal glucose tolerance in the study. Therefore, in the study of Telejko et al., a difference in serum apelin levels and a relationship between apelin and GDM could not be found between the study groups. In the same study, subcutaneous-visceral adipose tissue and placental tissue sampling of 20 pregnant women with GDM and 16 pregnant women with normal glucose tolerance were performed in the peripartum period, and apelin mRNA levels in the tissues were measured. While there was no difference between pregnant women with GDM and healthy pregnant women in terms of apelin mRNA levels in tissues, it was determined that the level of apelin mRNA in placenta tissue was ten times higher than in adipose tissue. In our study, serum apelin level was found to be higher in healthy pregnant women compared to healthy non-pregnant women. From this point of view, it is thought that the production of apelin from the placenta increases during pregnancy and plays a role in the regulation of placental blood flow and vascularity.

In various studies conducted in obese and type 2 diabetics, the relationship between serum apelin level and insulin resistance parameters has been revealed (16-17). In a study by Erdem et al., which included 40 newly diagnosed type 2 diabetes patients and 40 healthy adults, serum apelin levels were found to be significantly lower in the diabetic group, and a negative correlation was found between serum apelin levels and HOMA insulin resistance index (17). Similarly, in our study, serum apelin levels were found to be significantly lower in the pregnant group with GDM compared to the healthy pregnant group, and a negative correlation was observed between serum apelin levels and HOMA insulin resistance index.

The limitations of our study are that it is a cross-sectional study and the determination of serum apelin level during pregnancy with a single measurement. Also we do not know whether the decrease in apelin in pregnant women with GDM is due to increased clearance or decreased production. Visceral adipose tissue and/or placental tissue mass range may affect on apelin level in patients with GDM. Visceral fat tissue determination with dual energy x-ray absorptiometry or post-partum placental mass was not measured. Also the low number of the study population should be considered in our limitations. On the other hand, the research group includes healthy pregnant women and healthy women and pregnant women with GDM expresses its methodological strength.

In conclusion, low serum apelin levels are associated with the development of GDM. More studies are needed to show if serum levels of apelin precede the development of GDM, and if it can predict GDM development early in pregnancy.

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