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Maternal serum xenin-25 levels in gestational diabetes mellitus

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Abstract. – OBJECTIVE: Xenin-25 is a polypeptide having an insulinotropic effect *via* increasing the insulin-releasing action of glucose-dependent insulinotropic polypeptide (GIP) and promoting beta cell survival and proliferation. We aimed to assess serum xenin-25 levels in euglycemic pregnancies *vs.* pregnancies complicated with gestational diabetes mellitus (GDM).

PATIENTS AND METHODS: Forty pregnancies complicated with GDM and 40 healthy pregnancies with gestational age between 24 and 28 weeks were included. Following eight hours of fasting, blood samples were drawn from the participants, and subsequently, 75 g of glucose was administered orally. Blood was drawn again 120 minutes after the glucose challenge. Serum xenin-25 levels were detected by ELISA. Statistical analysis was performed, and $p<0.05$ was considered statistically significant.

RESULTS: There was no significant difference in maternal age, gestational age, BMI, fasting glucose, and insulin levels between the groups. Both fasting and 120th-minute xenin-25 levels were significantly higher in the GDM group when compared to the control group ($p<0.05$). Both the fasting and 120th-minute serum xenin-25 levels were significantly higher in women with GDM compared to healthy pregnant women.

CONCLUSIONS: High levels of xenin-25 were associated with gestational diabetes, and xenin-25 might be a potential marker for detecting GDM in the future.

Key Words:
Xenin-25, Gestational diabetes mellitus, Glucose, Pregnancy, Oral glucose tolerance test.

Introduction

Gestational diabetes mellitus (GDM) is the new onset of abnormally high serum glucose concentration during pregnancy[1]. Diabetogenic placental hormones like human placental lactogen and progesterone increase insulin resistance during pregnancy. In healthy pregnancies, glucose levels are regulated by pancreatic B-cell hyperplasia and increased insulin secretion, whereas this regulation cannot be achieved in pregnant women with GDM[2]. The adverse effects of GDM deteriorate maternofetal well-being and increase risks of preeclampsia, polyhydramnios, preterm labor, macrosomic newborn, shoulder dystocia, and intrauterine demise[3-5]. Therefore, screening and management of GDM are crucial since its prevalence is 9% in the USA, 2-6% in European countries, and 4.9% in Turkey[2,6,9].

Many endocrine mediators and their association with GDM have been investigated[7,8]. Xenin-25 is a neurotensin-like peptide, secreted simultaneously with glucose-dependent insulinotropic polypeptide (GIP) from duodenal enteroendocrine intestinal K cells. It is degraded quickly and has an insulinotropic effect. Xenin-25 increases the insulinotropic effect of existing GIP and directly increases insulin secretion. Other functions of xenin-25 are to slow down gastric motility in the duodenum, regulate pancreatic exocrine glands, and regulate the proliferation and survival of beta cells of pancreatic islets[10-12]. It also suppresses hunger at the ventromedial hypothalamic site. Thus, xenin-25 has a role in glucose homeostasis, and xenin-25 mechanisms are altered in type 2 diabetic patients[13]. Xenin-25 protein has homologs in procaryotes and fish at a similarity of 40% and 100%, however, evolutionarily, its importance has not yet been elucidated[12].

This study aimed to compare maternal serum xenin-25 levels between euglycemic pregnancies and pregnancies complicated with gestational diabetes mellitus.

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Patients and Methods

This study was designed as a prospective randomized study. A total of 80 pregnant women aged between 18 and 45, with a gestational age range between 24 and 28 weeks, were recruited. This study took place in the Obstetrics and Gynecology Clinics of Van Yuzuncu Yil University, between November 2019 and June 2020. The volunteers in this study were pregnant women who applied for a 75 g oral glucose tolerance test and who were without any known comorbidities. The study group involved 40 pregnant women who were diagnosed with GDM following the 2013 diagnostic criteria of the World Health Organisation[14]. GDM diagnosis of the participants was performed according to the results of the 75 g Oral Glucose Tolerance Test (OGTT). Pregnant women who had one of the following three criteria were considered as having GDM: having a fasting blood glucose (FBG) level of ≥92 mg/dl in the morning after an 8-hour nighttime fast and/or having a serum glucose level of ≥180 mg/dl at the 1st hour and/or having a serum glucose level ≥153 mg/dl at the 2nd hour after a 75 g oral glucose administration[14]. Multiple pregnancies, pregnancies with malabsorption disorders, pancreatic disease, surgery history including the gastrointestinal tract, pregestational diabetes, having a body mass index greater than 30 kg/m^2, or being on drugs affecting glucose regulation (corticosteroids, beta-agonists, etc.) were excluded. The control group of 40 pregnancies consisted of women who were maternal and gestational age-matched to the study group.

Biochemical Analysis

Maternal venous blood samples obtained for detection of serum xenin-25 levels in this study were the same samples drawn after 8 hours of fasting overnight and 2 hours after 75 g oral glucose administration. Blood samples were centrifuged at 3,000 rpm for 10 minutes, and serum samples were stored at -40°C until analysis. Serum glucose and insulin levels were measured in an Architect ci16200 biochemistry autoanalyzer (Abbott, Chicago, IL, USA) using commercial kits (Abbott, IL, USA).

Serum xenin-25 levels were measured with an enzyme-linked immunosorbent assay (ELISA) method, using a commercial ELISA kit manufactured by Shanghai YL Biotech Co. Ltd (catalog number: YLA4273HU; Shanghai, China). The intra- and interassay coefficients of variation were <8% and <10%, respectively. This assay has high sensitivity and excellent specificity for detecting human xenin-25. The values were expressed in units of pg/ml.

Statistical Analysis

Statistical analysis of the study was performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA). The distribution of parameters was analyzed with the Shapiro-Wilk test. All parameters were summarized as mean±standard deviation. Independent samples *t*-test was applied to assess differences between two means. ROC analysis was used to determine sensitivity and specificity. $p<0.05$ was considered statistically significant.

Results

A total of 80 pregnancies were involved in this study. There was no significant difference in maternal age, gestational age, BMI, Hemoglobin A1c (HbA1c) levels, fasting insulin, and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) values (Table I).

The mean maternal fasting glucose level was not significantly different between the groups. Sixty-minute and 120th-minute mean maternal glucose levels were significantly higher in GDM pregnancies (Table II).

Table I. Characteristics and glucose metabolism parameters of the participants.

Parameter	TGDM Group (n=40) mean±sd	Control Group (n=40) mean±sd	p
Maternal age (years)	33.1±4.9	30.1±7.3	0.193
Gestational age (weeks + days ± days)	25w+3d±9d	25w+4d±8d	0.771
Body mass index (kg/m^2)	28.3±1.3	27.9±1.7	0.687
HbA1c (%)	5.1±1.1	5.0±2.4	0.784
Fasting insulin (mIU/ml)	5.3±0.5	5.2±0.5	0.200
HOMA-IR	2.6±2.2	1.6±0.9	0.119

sd: standard deviation, w: week, d: day, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, HbA1c: Hemoglobin A1c.

Table II. Serum glucose and xenin levels of the participants during the oral glucose tolerance test.

Parameter	TGDM Group (n=40) mean±sd	Control Group (n=40) mean±sd	p
Serum glucose level at 0 min of OGTT (mg/dl)	86.1±15.9	80.1±18.9	0.149
Serum glucose level at 60 min of OGTT (mg/dl)	191.7±37.2	140.9±31.8	**<0.001**
Serum glucose level at 120 min of OGTT (mg/dl)	150.3±29.4	119.6±34.9	**<0.001**
Serum xenin level at 0 min of OGTT (pg/ml)	59.1±45.2	25.5±19.3	**0.001**
Serum xenin level at 120 min of OGTT (pg/ml)	60.1±39.4	26.3±20.7	**<0.001**

OGTT: 75 g oral glucose tolerance test, min: minute, sd: standard deviation.

Both the fasting and 120th-minute maternal xenin-25 levels of pregnancies in the GDM group were significantly higher than the control group (Table II).

There was no significant difference between fasting and 120th-minute xenin-25 levels in pregnancies with GDM. It was the same as for healthy pregnancies.

The ROC curve for fasting and 120th-minute xenin-25 in predicting GDM is shown in Figure 1. The area under the ROC curve for fasting xenin-25 and 120th-minute xenin-25 was 0.717 and 0.734, respectively. The optimal limit value of fasting xenin-25 for detecting GDM was calculated as 41.33 pg/ml with a sensitivity of 64.5% and a specificity of 73.3%. The optimal level of 120th-minute xenin-25 for detecting GDM was calculated as 43.03 pg/ml with a sensitivity of 64.4% and a specificity of 72.2%.

Discussion

Gestational diabetes mellitus (GDM) is a condition where women develop varying degrees of carbohydrate intolerance during pregnancy or are diagnosed for the first time during pregnancy[15]. A normal pregnancy is a condition that progresses with insulin resistance, hyperinsulinemia, and mild postprandial hyperglycemia under the influence of diabetogenic hormones. This situation

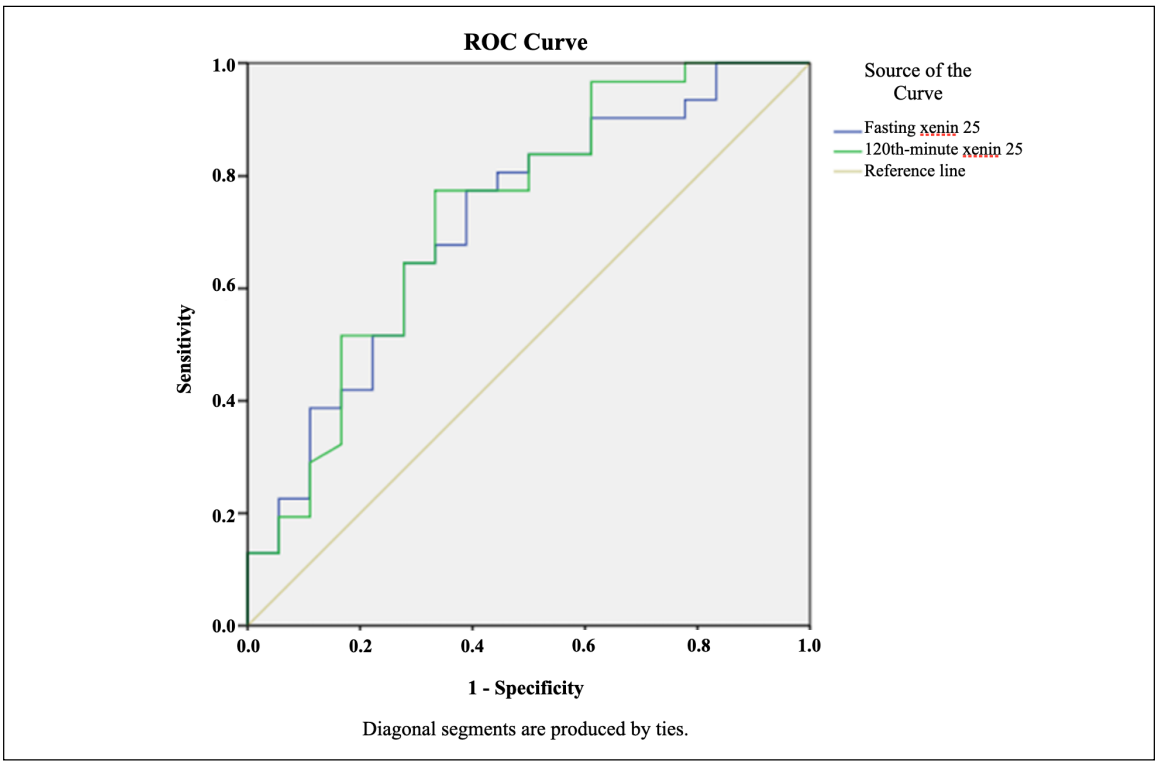

Figure 1. Receiver operating characteristic (ROC) curve for xenin-25 for the prediction of GDM.

prepares the mother to meet the increased amino acid and glucose needs of the fetus, particularly in the second half of pregnancy[15]. The decrease in insulin sensitivity that occurs during normal pregnancy leads to GDM. GDM develops when maternal pancreatic β cells do not secrete sufficient insulin to meet the increased insulin need[16].

Xenin-25 K cell-derived incretin hormone is released into the bloodstream along with GIP and is known to enhance the biological effects of GIP[17]. Since the insulin-releasing effect of GIP is compromised in type 2 diabetes mellitus, this effect of xenin-25 on GIP is important in terms of being a therapeutic drug target for type 2 diabetes mellitus[18]. The effects of xenin-25 on GIP-mediated insulin and glucagon are greatest in glucose-tolerant people[19]. Similarly, xenin has been shown to have a greater effect on GIP-mediated insulin secretion in hyperglycemic mice compared to normoglycemic mice[20]. These results indicate that increased cholinergic signals are a compensatory neural mechanism that increases insulin secretion before diabetes, and type 2 diabetes develops if this adaptation fails[21].

A study[22] that was conducted with adolescents demonstrated that the xenin-25 concentration was higher in obese adolescents compared to healthy adolescents. It was found in a study that was conducted on women with polycystic ovary syndrome (PCOS), that the xenin concentration was significantly higher among women with PCOS compared to the healthy control group[23].

Our study was performed with a total of 80 pregnant women whose characteristic parameters (maternal age, gestational age, BMI, HbA1c levels, fasting insulin, fasting glucose, and HOMA-IR values) were matched in order to compare xenin-25 levels between GDM pregnancies and euglycemic pregnancies. As was expected, the study group pregnancies had higher levels of the 60th-minute and 120th-minute serum glucose after 75-OGTT.

In the presented study, both fasting and 120th-minute xenin-25 levels were found to be significantly and approximately 2-fold higher in the GDM group compared with healthy pregnant women. The higher amount of xenin-25 might represent the additional need for insulinotropic action to lower serum glucose levels in the diabetic milieu. The increased levels of xenin-25 would increase the potential of GIP, which has an insulin-releasing action. An increase in xenin-25 would also promote beta cell survival and proliferation, which would be vital for carrying on higher insulin secretion with decreasing beta cell loss.

The cut-off value to detect GDM pregnancies was a fasting xenin-25 level of 41.33 pg/ml with a sensitivity of 64.5% and a specificity of 73.3%, whereas the 120th-minute xenin-25 cut-off value was 43.03 pg/ml with a sensitivity of 64.45% and a specificity of 72.2%. In a study conducted with PCOS patients, the cut-off value of xenin-25 for PCOS was reported to be 32.60 pg/ml with a sensitivity of 61.3% and a specificity of 86.7%, with a probability of 4.5[23].

It was shown that xenin-25 was effective in glucose homeostasis in normal and impaired glucose tolerance but not in type 2 diabetes[19]. Although the two groups had similar mean fasting insulin levels, the GDM group had higher fasting xenin-25 levels, which was puzzling. As was shown for insulin resistance, xenin-25 levels might have increased in order to supply the same amount of serum insulin by various mechanisms in order to regulate glucose metabolism.

Limitations

This study had some limitations. In this study, we only measured fasting insulin. Future research may be designed to investigate the measurement of both insulin and GIP during fasting and at the 60th minute and 120th minute of OGTT. According to the study protocol, BMI was calculated only at the time of measurement, however, in subsequent studies, weight gain during pregnancy could also be taken into account. In addition, xenin-25 levels were measured at 24-28 weeks of pregnancy, and a better evaluation could be made by periodically measuring the xenin-25 level at certain weeks from the beginning of pregnancy to the OGTT process.

Conclusions

In this study, fasting and 120th-minute serum xenin-25 levels were found to be significantly higher in pregnant women with GDM compared to healthy pregnant women. Serum xenin-25 level may be a candidate for the diagnosis of GDM. The potential and function of this ancestral protein need to be studied as a participant in glucose metabolism, and whether it is diagnostically strong enough to predict GDM. In order to provide this information, future studies should be conducted in larger populations to better understand the relationship between GDM and xenin-25.

Ethics Approval
The study protocol was appropriate to the Declaration of Helsinki, and ethical approval was obtained from the Ethical Committee of Van Yuzuncu Yil University (Approval Date/Number: 20.11.2019/010).

Informed Consent
Informed consent was obtained from all subjects involved in the study.

Conflict of Interest
The authors declare no conflict of interest.

Data Availability
The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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This research received no external funding.

Authors' Contributions
Conceptualization, GNK, DD, and HGS; methodology, OK, CK, BK, and YD; software, AUK, and YD; validation, BK, CK, OK, and YD; formal analysis, AUK, DD, OK, and HGS; investigation, GNK, DD, and HGS; data curation, GNK, DD, and AUK; writing-original draft preparation, GNK, DD and AUK; writing-review and editing, GNK and HGS; visualization, GNK, DD, and AUK; supervision, HGS, and AUK; project administration, HGS.

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