A novel marker endoplasmic reticulum to nucleus signalling-1 in the diagnosis of gestational diabetes mellitus

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Abstract

Objective: We aimed to investigate maternal plasma endoplasmic reticulum to nucleus signalling-1 (ERN-1) concentrations in patients diagnosed with gestational diabetes mellitus (GDM).

Material and Methods: This was a cross-sectional study of 57 pregnant women with GDM and 40 gestational age- and body mass index-matched, healthy pregnant controls, conducted between August 2020 and November 2020. Plasma ERN-1 levels, other laboratory markers of insulin resistance, and demographic characteristics were compared between groups.

Results: Fasting glucose, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), hemoglobin A1c and plasma ERN-1 levels were significantly higher in the GDM group than in the healthy controls ($p<0.001$). Positive correlation was found between ERN-1 levels and HOMA-IR values ($p=0.016$). The optimal cut-off value for ERN-1 to diagnose GDM was 6.960 ng/mL, with a sensitivity of 78.9% and a specificity of 75.0% ($p<0.001$).

Conclusion: ERN-1 may be considered as a new marker for diagnosis of GDM and may also be a potential target in studies of GDM treatment modalities. (J Turk Ger Gynecol Assoc 2022; 23: 106-10)

Keywords: Endoplasmic reticulum stress, endoplasmic reticulum to nucleus signalling-1, getational diabetes mellitus, unfolded protein response

Received: 30 September, 2021 **Accepted:** 21 December, 2021

Introduction

Gestational diabetes mellitus (GDM) is traditionally described as abnormal glucose tolerance with onset, or noticed for the first time, in the course of gestation (1,2). GDM develops when pancreatic ß-cells insulin secretion is unable to compensate for physiologic insulin resistance of pregnancy because of impaired ß-cell function (3-5). Although it differs based on the population characteristics and diagnostic criteria, GDM is a complication that occurs in about 6-7% of pregnancies. However, it is known that the frequency of GDM has tended to increase in the last years because of the increasing trends in the average maternal age and obesity (6-8). GDM with uncontrolled blood glucose levels can lead to some complications for the mother and baby, including macrosomia, shoulder dystocia, birth trauma, preeclampsia, cesarean delivery, neonatal hyperbilirubinemia, and hypoglycemia (9-11). These women are also at increased risk of developing type 2 diabetes, cardiovascular disease, and metabolic syndrome in the future. Furthermore, children born to a woman with GDM have increased risks for obesity and diabetes later in life (12).

Increasing evidence suggests that endoplasmic reticulum (ER) stress is strongly related to the pathogenesis of diabetes. The ER is an organelle in which newly synthesized secretory proteins

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.galenos.2021.2021-9-28

are folded and assembled. Peripheral insulin resistance can induce pancreatic ß-cells to increase production of proinsulin. Overproduction of proinsulin leads to excessive unfolded or misfolded proinsulin accumulation in the ER. When this accumulation becomes intolerable, ß-cell toxicity death occurs. This is known as ER stress. In an attempt to ameliorate the ER stress, a defense system known as the "unfolded protein response" (UPR) is activated in ß-cells (13-15). UPR signaling is regulated by three main molecules: endoribonuclease inositol-requiring protein-1α (IRE-1α); activating transcription factor-6; and protein kinase RNA-like endoplasmic reticulum kinase (16). IRE-1α is encoded by the *ERN-1* gene in humans and is also known as ER to nucleus signalling-1 (ERN-1). Since the pathophysiology of GDM is similar to type 2 diabetes, we hypothesized that ERN-1 levels may be altered in patients with GDM, secondary to increased ER stress and UPR activation.

In this study, we investigated circulating levels of ERN-1 in patients with GDM and compared these with healthy pregnant controls. To the best of our knowledge, this is the first study to examine circulating levels of ERN-1 and its utility in the diagnosis of GDM.

Material and Methods

Patient selection

This cross-sectional study was performed in the Perinatology outpatient clinic of the University of Health Sciences Turkey, Kanuni Sultan Süleyman Training and Research Hospital, between January 2020 and May 2020. The study was approved by the University of Health Sciences Turkey, Kanuni Sultan Süleyman Training and Research Hospital Clinical Research Ethics Committee (approval number: KAEK/2020.07.159). Written informed consent was obtained from all participants.

Singleton pregnant women between 24 and 28 gestational weeks were eligible for participation. The GDM group consisted of pregnant women diagnosed following an oral glucose tolerance test (OGTT) while the healthy control group of randomly selected gestational week- and body mass index (BMI)-matched healthy pregnant women had normal results on OGTT. GDM screening was performed with a 75 g OGTT in all participants. According to the International Association of Diabetes and Pregnancy Study Groups criteria, GDM was diagnosed if at least one of the following plasma glucose levels (fasting ≥92 mg/dL, 1 hour ≥180 mg/dL, 2 hour ≥153 mg/dL) were obtained (1). In patients diagnosed with GDM, diet modification was made and insulin therapy was started, if necessary.

Patients who had pre-existing diabetes mellitus, chronic hypertension or gestational hypertensive disorders, and multiple gestation were excluded.

Blood sampling

Fasting venous blood samples were taken from patients to analyze ERN-1 concentrations. After clotting, the samples were immediately centrifuged at 3000 rpm for 10 minutes. Serum was separated and stored at -80 °C until analysis. A commercial ELISA kit was used for the quantitative analysis of ERN-1 levels (Mybiosource Inc., San Diego, CA, USA. Catalog No: MBS1603218).

For secondary comparative analyses, insulin and hemoglobin A1c (HbA1c) concentrations were analyzed. The homeostatic model assessment of insulin resistance (HOMA-IR) values were calculated with the following equation: fasting glucose (mmol) x fasting insulin (lU/mL)/22.5.

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL, United States). Kolmogorov-Smirnov and Shapiro-Wilk tests were used to analyze the distribution of continuous variables. The Levene test was used to analyze the homogeneities of variances. Chi-square and/or Fisher's exact test for categorical variables and Student's t-test or Mann-Whitney U test for continuous variables were used to evaluate differences between groups. Correlations between variables and ERN-1 were evaluated by Spearman's correlation coefficient and related p-values. ERN-1 cut-off value was estimated by using the index of Youden. A $p<0.05$ was considered as statistically significant. Post-hoc analysis was conducted for ERN-1, which was determined as the primary outcome variable of the study, in order to find a statistically significant difference of approximately 20% between the control and GDM groups means, for which the sample size was calculated according to the F-test with 0.05 error level and minimum 80% power. The sample size was found to be 90.

Results

We included a total of 97 singleton pregnant women, of whom 57 (58.8%) were diagnosed with GDM and the remaining 40 (41.2%) were controls with normal OGTT results. Among the 57 women with GDM, while glycemic control could be achieved in 35 (61.4%) by diet, insulin therapy was started in 22 (38.6%) of them. Demographic features and clinical outcomes of patients with GDM and healthy controls are shown in Table 1. There was no significant difference between the groups in terms of maternal age, height, weight, BMI, gestational week at sampling, gestational week at birth and birth weight. Biochemical parameters of the two groups are presented in Table 2. Fasting Glucose, insulin, HbA1c, HOMA-IR and ERN-1 variables were compared between the GDM and control group

and all were significantly higher in the GDM group compared to the control group (all; p<0.001).

Correlations between maternal ERN-1 levels and other clinical or biochemical variables were analyzed. No correlation was observed with any parameter other than HOMA-IR where there was a positive correlation with ERN-1 levels (r=0.329, p=0.016; Table 3).

According to the receiver operating characteristic analysis, ERN-1 level was a statistically significant parameter to predicting GDM. The cutoff value was 6.960 ng/mL for the diagnosis of GDM with a sensitivity of 78.9% and a specificity of 75.0% (p<0.001) (Figure 1).

Discussion

In this study, the plasma ERN-1 levels, demographic characteristics and biochemical parameters of women with GDM and normoglycemic pregnant controls were compared. ERN-1 concentrations in patients with GDM were significantly higher than those of the healthy control group. Furthermore, serum ERN-1 concentrations were significantly positively correlated with HOMA-IR values. HOMA-IR values are derived from measurements of fasting insulin and fasting glucose values but, interestingly, the only correlation identified was between ERN-1 and the final HOMA-IR value while no correlation was observed with fasting insulin and fasting glucose. We suggest that plasma ERN-1 concentrations may be a novel and predictive parameter for GDM.

The pathogenesis of diabetes is not completely understood but growing evidence suggests that, during progression of disease, loss of pancreatic ß-cell function and ultimate loss of ß-cell mass is accompanied by ER stress (13-15). Pancreatic ß-cells increase proinsulin production as a result of insulin-resistant peripheral tissues. The ER protein-folding mechanisms may be overwhelmed with the excess production of proteins, including proinsulin, translocated into the ER, resulting in ER stress. These unfolded proteins aggregate in the ER and activate downstream signaling mechanisms, which has been called the UPR (16). The UPR leads to protein translation attenuation and also triggers a mechanism to remove misfolded protein from the ER, which has been termed ER-associated degradation (17). IRE-1 acts as an ER stress sensor of unfolded proteins in the ER and triggers UPR. ERN-1 protein is a human homologue of the IRE-1.

Type 2 diabetes and GDM have similar physiopathological mechanisms, notably insulin resistance. Therefore, we hypothesized that ERN-1 concentrations may increase in the course of GDM, as they do in type 2 DM, secondary to increased ER stress and UPR. This study has demonstrated that ERN-1 concentrations were substantially increased in patients with GDM and provided evidence that increased ER stress may also perform a role in the pathophysiology of GDM, similar to type 2 diabetes.

Conclusion

This study is the first to report that maternal ERN-1 levels are significantly higher in patients with GDM than in healthy, gestational week- and BMI-matched pregnant women.

Table 1. Demographic features and clinical outcomes of the groups

	GDM group (n=57)	Control group (n=40)	p
Age (years)	32.40±6.15	30.62±3.79	0.108*
Maternal height (cm)	161.03±5.70	162.37±4.49	0.219*
Maternal weight (kg)	74.14±10.94	71.80±6.14	0.564
Body mass index (kg/m^2)	28.52±3.80	27.21±1.86	0.158
Gestational week at sampling	27.43±3.33	26.35±2.60	0.060
Gestatioanl week at birth	38.77±2.764	39.12±2.20	0.588
Birth weight (g)	3226.70±721.95	3254.55±346.03	0.424
*Asterisked p-values refer to p-values from Student's t-test, others to Mann-Whitney U test. GDM: Gestational diabetes mellitus			

Table 2. Biochemical parameters of women diagnosed with GDM and the control group

	GDM group (n=57)	Control group (n=40)	p
Fasting glucose (mg/dL)	95.36±13.26	80.20±5.73	**<0.001***
Insulin (mU/L)	12.87±3.04	8.05±1.75	**<0.001***
HbA1c (%)	5.93±0.87	5.05±0.33	**<0.001**
HOMA-IR	3.03±0.80	1.58±0.33	**<0.001**
ERN-1 (ng/mL)	9.70±5.24	6.39±1.53	**<0.001**
*Asterisked p-values refer to p-values from Student's t-test, others to Mann-Whitney U test. GDM: Gestational diabetes mellitus, ERN-1: ER to nucleus signalling-1, HbA1c: Hemoglobin A1c, HOMA-IR: Homeostasis model assessment of insulin resistance			

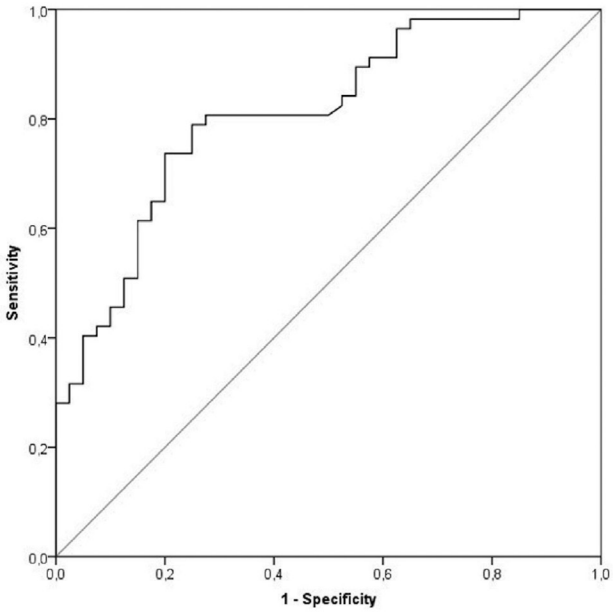

Figure 1. Receiver operating characteristic curve showing the diagnostic utility of ER to nucleus signalling-1 in gestational diabetes mellitus

Table 3. Correlation analyses between ERN-1 concentration and other variables

ERN-1		
	r	**p**
Age	-0.322	0.083
Maternal height	-0.021	0.912
Maternal weight	0.168	0.375
BMI	0.231	0.220
HbA1c (%)	0.033	0.864
Insulin (mU/L)	0.240	0.202
Fasting glucose (mg/dL)	0.047	0.804
HOMA-IR	0.329	0.016
Gestatioanl week at birth	-0.194	0.304
Birth weight (g)	-0.088	0.644
Gestational week at sampling	0.136	0.233

ERN-1: ER to nucleus signalling-1, BMI: Body mass index, HbA1c: Hemoglobin A1c, HOMA-IR: Homeostasis model assessment of insulin resistance

Furthermore, ERN-1 concentration was positively correlated with HOMA-IR. Prompt recognition and management of GDM are essential to reduce the adverse fetal and maternal outcomes and protecting neonates and mothers from long-term complications. The OGTT, which is widely used for screening pregnant women at risk of GDM, is not comfortable and tolerable by many pregnant women. In this context, ERN-1 might be suggested as a potential marker to predict the development of GDM. ERN-1 should also be considered as a potential target for medical intervention in GDM.

Ethics Committee Approval: *The study was approved by the University of Health Sciences Turkey, Kanuni Sultan Süleyman Training and Research Hospital Clinical Research Ethics Committee (approval number: KAEK/2020.07.159).*

Informed Consent: *Written informed consent was obtained from all participants.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: S.S.Ç., M.B.; Concept: S.S.Ç., M.B.; Design: S.S.Ç., M.B.; Data Collection or Processing: S.S.Ç., M.B.; Analysis or Interpretation: S.S.Ç., M.B.; Literature Search: S.S.Ç., M.B.; Writing: S.S.Ç., M.B.*

Conflict of Interest: *No conflict of interest is declared by the authors.*

Financial Disclosure: *The authors declared that this study received no financial support.*

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