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Research

Predictive Value of Plasma Zonulin Levels during Early Gestational Weeks in Gestational Diabetes Mellitus

Erken Gestasyonel Haftalarda Değerlendirilen Plazma Zonulin Düzeylerinin Gestasyonel Diabetes Mellitus İçin Prediktif Değeri

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ABSTRACT

Objective: This study aimed to determine the impact and predictive utility of serum zonulin levels measured between 11-14 weeks of gestation and 75-g oral glucose tolerance test (OGTT) testing between 24-28 weeks of gestation in predicting patients diagnosed with gestational diabetes mellitus (GDM).

Methods: The study included 209 pregnant women at 11-14 weeks of gestation. Among the pregnant women who presented to the Obstetrics and Gynecology Outpatient Department of İstanbul Medipol Mega Hospital, those who could not be reached due to the covid pandemic (69), aborted (4), diagnosed with chromosomal abnormalities in the fetus (3), and those who withdrew from the study (4) were excluded from the study. The study group included 48 pregnant women diagnosed with GDM at 24-28 weeks of gestation. On the other hand, the control group was formed by randomly selecting 40 pregnant women from 81 pregnant women who were not diagnosed with GDM due to the lack of a kit using a computer-based program. GDM was diagnosed using an OGTT performed between 24 and 28 weeks of gestation according to the International Association of Diabetes and Pregnancy Study Groups criteria. Plasma zonulin levels were measured using enzyme-linked immunosorbent assay.

Results: When zonulin (ng/mL) and body mass index (BMI) (kg/m^2) values were compared between the study and control groups, a significant correlation was found between zonulin (35.77±8.79 (ng/mL), 29.76±6.96 (ng/mL), p=0.01) and BMI (kg/m^2) (26.02±2.39, 24.78±2.7 (kg/m^2), p=0.032). In addition, a correlation analysis showed a significant positive correlation between plasma zonulin level and first-hour OGTT.

Conclusion: The findings of our research indicate that zonulin has the potential to function as a non-invasive biomarker of GDM development. More extensive research is required on this topic.

Keywords: Body mass index, gestational diabetes mellitus, zonulin

ÖZ

Amaç: Bu çalışmada, 11-14. gebelik haftaları arasında ölçülen serum zonulin düzeylerinin ve 24-28. gebelik haftaları arasında yapılan 75 g oral glukoz tolerans testi (OGTT)'nin gestatsyonel diyabetes mellitus (GDM) teşhisi konulan hastaları öngörmedeki etkisini belirlemeyi amaçladık.

Gereç ve Yöntem: Çalışmaya 11-14 gebelik haftasındaki 209 gebe kadın dahil edilmiştir. İstanbul Medipol Mega Hastanesi Kadın Hastalıkları ve Doğum Polikliniğine başvuran bu gebelerden covid pandemisi nedeniyle ulaşılamayanlar (69), abortus yapanlar (4), fetüste kromozomal anormallik saptananlar (3) ve çalışmadan çekilenler (4) çalışma dışı bırakılmıştır. Çalışma grubuna 24-28. gebelik haftalarında GDM tanısı alan 48 gebe kadın dahil edildi. Buna karşılık, kontrol grubu ise GDM teşhisi konmamış 81 gebe içerisinden kit yetersizliğinden dolayı 40 gebe kadın bilgisayar tabanlı program kullanılarak rastgele seçilerek oluşturulmuştur. GDM tanısı, Uluslararası Diyabet ve Gebelik Çalışma Grupları Birliği kriterlerine göre 24-28. gebelik haftaları arasında yapılan bir OGTT ile konulmuştur. Plazma zonulin düzeyleri enzime bağlı immünosorbent testi ile ölçülmüştür.

Bulgular: Çalışma ve kontrol grupları arasında zonulin (ng/mL) ve vücut kitle indeksi (VKİ) (kg/m^2) değerleri karşılaştırıldığında, zonulin (35.77±8.79 (ng/mL), 29.76±6.96 (ng/mL), p=0.01) ve VKİ (kg/m^2) (26.02±2.39, 24.78±2.7 (kg/m^2), p=0.032) arasında anlamlı bir korelasyon bulunmuştur. Ayrıca, korelasyon analizi plazma zonulin düzeyi ile birinci saat OGTT arasında anlamlı bir pozitif korelasyon olduğunu göstermiştir.

Sonuçlar: Araştırmamızın bulguları, zonulinin GDM gelişimi için non-invaziv bir biyobelirteç olarak işlev görme potansiyeline sahip olduğunu göstermektedir. Bu konuda daha geniş ölçekli çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Gestasyonel diabetes mellitus, vücut kitle indeksi, zonulin

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INTRODUCTION

Gestational diabetes mellitus (GDM) is a medical condition characterized by impaired tolerance to carbohydrates, which is observed specifically during pregnancy (1-4). Not only the infant but also the mother exhibits heightened vulnerability to complications during and after pregnancy. A global consensus on the diagnosis, screening, and treatment of GDM is yet to be reached. Added to this, there also remains a lack of consensus regarding the optimal timing for conducting screening, the appropriate thresholds to employ in screening, the population to target for screening (i.e., all pregnant women or solely those at high risk of GDM), and the preferred approach to the screening test (i.e., one-stage or two-stage) (5-9).

Zonulin serves as a significant noninvasive biomarker that indicates heightened intestinal permeability, and increased levels of zonulin indicate heightened impairment in the functioning of the intestinal barrier (10). Numerous studies have shown a correlation between increasing intestinal barrier dysfunction and elevated inflammatory markers [tumor necrosis factor, interleukin-6 (IL-6), etc.] and insulin resistance in women who are not pregnant (11, 12). Serum zonulin has been shown to correlate with dietary composition, suggesting a novel pathway through which diet could influence the development of metabolic disorders associated with inflammation (13). However, very few studies have investigated the relationship between GDM and zonulin (13, 14). To the best of our knowledge, this is one of the first studies to correlate circulating zonulin concentration (a marker and modulator of intestinal permeability) in patients in terms of risk groups and screening time for GDM.

In this study, we aimed to determine the impact and predictive utility of serum zonulin levels measured between 11-14 weeks of gestation and 75-g OGTT testing between 24-28 weeks of gestation in predicting patients diagnosed with GDM.

METHODS

Establishment of study groups

This study was conducted with the approval of the Ethics Committee for Noninterventional Research of Wma Declaration Of Helsinki-Ethical Principles Formedical Research Involving Human Subjects (date: 20.08.2020, decision number: 626). Following the distribution of the informed consent form to all participants, the purpose of the study was shared with them to secure their voluntary participation in the research. The cases in our study consisted of pregnant women who presented to our outpatient clinic at the Department of Obstetrics and Gynecology, İstanbul Medipol Mega Hospital Hospital between 20/08/2020 and 15/01/2022.

The study included 209 pregnant women at 11-14 weeks of gestation. Among these pregnant women, those who could not be reached due to the covid pandemic (69), aborted (4), diagnosed with chromosomal abnormalities in the fetus (3), and those who withdrew from the study (4) were excluded from the study. During the gestational period spanning from the 24th week to the 28th week, a total of 129 women were subjected to a 75-g oral glucose tolerance test (OGTT). The study group consisted of 48 pregnant women diagnosed with GDM based on the diagnostic criteria established by the International Association of Diabetes and Pregnancy Study Groups. Due to the lack of available kits, only 40 out of the total 81 pregnant women who had not received a GDM diagnosis were randomly allocated to the control group.

The Blood Sample Collection Method

Women presenting to the obstetrics and gynecology outpatient clinic at İstanbul Medipol University Hospital were asked to observe a minimum fasting period of 8 hours. Following the acquisition of informed consent, a 5-cc blood sample was collected from pregnant women during the 11th to 13th weeks of gestation to measure zonulin levels. The blood was drawn into biochemical tubes and subsequently centrifuged at 3000 rpm. The plasma samples were collected and then stored at a temperature of -80 ⁰C until the day of analysis.

Serum Zonulin Level Determination

Serum zonulin levels were determined by the ELISA (Enzyme-Linked Immunabsorbent Assay) method (Elabscience, catalog number: E- EL -H5560, Wuhan, Hubei Province, China). The coefficients of variation for both intra-assay and inter-assays were 10%.

Statistical Analysis

The mean and standard deviation were used to express continuous quantitative variables, whereas qualitative variables were expressed using n and median. Normality tests for the variables were conducted using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The Mann-Whitney rank sum test was used to analyze continuous variables comprising independent variables that did not exhibit a normal distribution. Receiver operating characteristic curve analysis was applied to determine values such as cut-off point, sensitivity, and specificity of continuous variables. Spearman's rho correlation test was used to determine the

degree of relationships between variables. We attributed significance to probability values less than 0.05. All data analyses were performed using the IBM SPSS Statistics 21 package.

RESULTS

The mean standard deviation (unit) of the parameters studied among the pregnant women who participated in the study were age (28.875±3,45 years), BMI (25.45±2.59 (kg/m^2), fasting plasma glucose (FPG) (90.3±9.3 (mg/dL), OGTT 1st hour (163.16±32.877 (mg/dL), OGTT 2nd hour (123.53±24 (mg/dL), and zonulin (33.04±8.5 (ng/mL). 60.2% of the participating pregnant women were first-time pregnancies. Table 1 and Figure 1 present the standard deviation values for zonulin (ng/mL), age (years), BMI (kg/m^2), FPG (mg/dL),

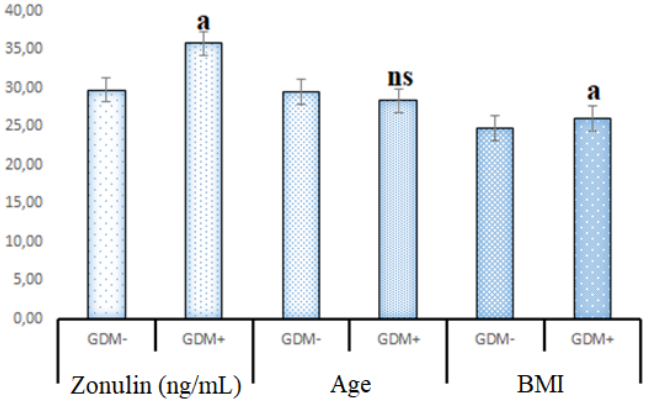

Figure 1. Zonulin, Age, and BMI of the study group (S) and control group (C)
BMI: Body mass index, GDM: Gestational diabetes mellitus

first-hour OGTT (mg/dL), and second-hour OGTT (mg/dL) in both the study and control groups. Serum zonulin levels were elevated in patients with GDM compared with the control group, which was determined to be of statistical significance (p<0.01). Although no significant correlation was found between age and GDM (p=0.645), there was a significant correlation was found between BMI (kg/m^2) and GDM (p=0.032). No statistically significant correlation was observed between gravidity and parity (p=0.148). However, a statistically significant difference was observed between the study and control groups about FPG scores (p<0.001).

In the correlation analysis, a significant positive correlation was found between serum zonulin and the first-hour OGTT (mg/dL) (p=0.009, r=0.278), whereas no significant correlation was found between serum zonulin and other parameters (Table 2).

The Youden index, the optimal cutoff value for serum zonulin, was ≥36.783 (ng/mL) in predicting GDM between the 24th and 28th months of pregnancy, with a sensitivity of 54.17% and a specificity of 87.5%. The area under the receiver operating characteristic (ROC) curve was 0.706 (95% CI: 0.6-0.799) (Figure 2).

DISCUSSION

Zonulin is a novel circulatory marker of intestinal permeability (11, 15). Mokkala et al. (15) showed for the first time an association between increased serum zonulin levels in early pregnancy and GDM, suggesting that zonulin may serve as a predictive marker for GDM. In their study of women who were 12.8±2.5 weeks pregnant, they observed

Table 1. Demographic and laboratory characteristics of the control and gestational diabetes mellitus (GDM) groups

Groups	Control (GDM-)			Group Work (GDM+)			p-value
	N	Mean	SD	N	Mean	SD	
Zonulin (ng/mL)	40	29.76	6.96	48	35.77	8.79	<0.01
BMI (kg/m^2)	40	24.78	2.03	48	26.02	2.392	=0.032
Age	40	29.475	4.26	48	28.375	2.284	=0.645
FPG (mg/dL)	40	83.92	5.25	48	95.72	8.6	<0.001
OGTT (1.Hour) (mg/dL)	40	137.2	24.04	48	184.77	21.7	<0.001
OGTT (2.Hour) (mg/dL)	40	110.15	18.34	48	134.68	23.05	=0.550

BMI: Body mass index, FPG: Fasting plasma glucose, OGTT: Oral glucose tolerance test, SD: Standard deviation

Table 2. Correlation Analyses of the Study Groups

			Y	BMI (kg/m^2)	FPG (mg/dl)	OGTT 1.H. (mg/dL)	OGTT 2.H. (mg/dL)
Spearman's rho	Zonulin (ng/mL)	r	.048	.119	.150	.279**	.065
		p	.659	.269	.163	.009	.550

Significant difference compared with zonulin
**: p<0.001, BMI: Body mass index, FPG: Fasting plasma glucose, OGTT: Oral glucose tolerance test

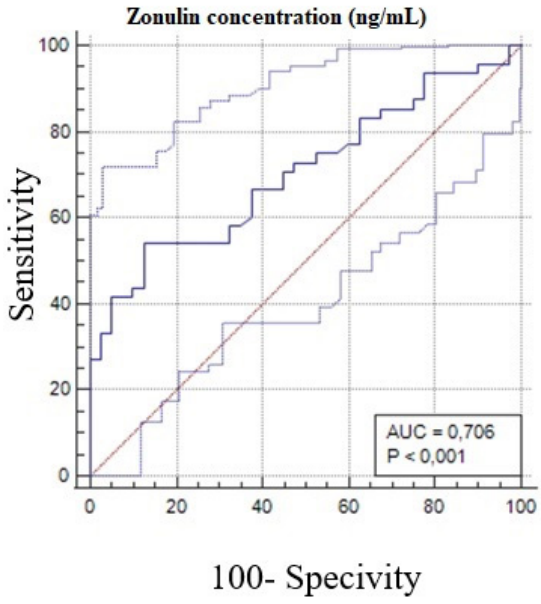

Figure 2. ROC curve for serum zonulin level for predicting GDM
ROC: Receiver operating characteristic, GDM: Gestational diabetes mellitus, AUC: Area under the curve

that those who developed GDM later in mid-pregnancy had higher serum zonulin levels in the early stages of pregnancy. Furthermore, a study using ROC curve analysis and the Youden index found that the optimal cutoff point for serum zonulin concentration to predict GDM in mid-pregnancy is ≥43.5 ng/mL, with a sensitivity of 88% (95% confidence interval (CI): 71-100%) and a specificity of 47% (95% CI: 33-58%) (15). The fact that the number of patients with GDM was only sixteen and the specificity score was low is a limitation of this study. Demir et al. (14) also reported elevated plasma zonulin levels in patients with GDM. The study population consisted of women between 24 and 28 weeks of pregnancy. They reported that in patients with plasma zonulin levels of 20 ng/mL, the sensitivity was 98.8%, specificity was 100%, positive predictive value (PPV) was 98.8%, and negative predictive value was 100%. They also found a significant correlation between plasma zonulin and the first hour of the OGTT ($p<0.001$, $r=0.568$) (14). Consistent with prior research (14, 15), our study found elevated plasma zonulin levels in pregnant women diagnosed with GDM. Furthermore, a remarkable positive correlation was found between plasma zonulin levels and the first hour of the OGTT ($p=0.009$, $r=0.279$). The differences observed in sensitivity and specificity may be accounted for by the timing of the test during pregnancy as well as the criteria for excluding participants from the study. The prevalence of pregnancy-related complications, including GDM, is progressively rising, as evidenced by previous studies. According to the results of our study, the sensitivity was 54.17% (95%) and the specificity was 87.5% (95%). Furthermore, the optimal threshold for serum zonulin concentration to predict the development of GDM between the 24th and 28th weeks of pregnancy was >36.783 (ng/mL).

Diet and lifestyle are significant risk factors for obesity and GDM. The observation that there has been a notable rise in the incidence of obesity among women in the age group capable of bearing children, reaching approximately 30%, and that a significant proportion of pregnant women face a heightened susceptibility to GDM should not be disregarded. Studies have suggested that the adoption of certain measures, including adhering to a nutritious diet before and during pregnancy, maintaining a BMI below 25 kg/m^2, engaging in physical activity exceeding 30 minutes per day, and refraining from smoking, can potentially reduce the incidence of GDM by approximately 45% (16), which offers the possibility of avoiding short- and long-term complications associated with GDM. Furthermore, the result of the measurement can serve expectant mothers as a valuable reference for adjusting their lifestyle.

Recent studies have indicated a correlation between dietary and serum zonulin concentrations (13). People who eat diets deficient in naturally occurring antioxidants, fiber, and omega-3 fatty acids from fruits, vegetables, and whole grains, while being abundant in refined starches, sugars, saturated fats, and trans fats, tend to have significantly elevated levels of pro-inflammatory cytokines and lower levels of anti-inflammatory cytokines (17). According to previous reports, the presence of chronic inflammation resulting from dietary habits in this particular situation can potentially contribute to adverse outcomes, including metabolic syndrome, obesity, cardiovascular disease (CVD), type 2 diabetes mellitus (DM), and cancer (18, 19). Previous studies have demonstrated a positive correlation between zonulin levels and the likelihood of experiencing inflammation, insulin resistance, elevated fasting blood glucose levels, and metabolic syndrome (11, 15, 20). A study by Mokkala et al. (15) revealed a correlation between metabolic endotoxemia, inflammation, glucose and lipid metabolism, and serum zonulin concentrations in obese pregnant women. The activation of metabolic processes by zonulin has deleterious effects on maternal and fetal health. By supporting the integrity of the intestinal barrier and modifying the composition of the diet, it is possible to prevent negative outcomes resulting from metabolic reactions (21). This objective can be achieved by adhering to a Mediterranean diet characterized by high fiber content (22), low glycemic load and index (23), and anti-inflammatory (24) and antioxidant properties (25). One

study found an association between plasma levels of zonulin and the consumption of various vitamins, minerals, omega-3 fatty acids, and dietary fiber, which affect the composition and diversity of the gut microbiota in obese pregnant women (13).

CONCLUSION

GDM is a risk factor with acute and long-term fetal and maternal effects in the intrapartum and postpartum periods. The maternal effects can manifest as type 2 DM, hypercholesterolemia, and elevated susceptibility to CVD, whereas the fetal effects can manifest as abnormalities, DM, and increased accumulation of adipose tissue. To mitigate the acute and long-term impacts of GDM on both the mother and fetus, it is crucial to prioritize early identification and intervention, which requires a multidisciplinary approach encompassing the diagnosis, treatment, and subsequent monitoring of GDM. In the present study, the optimal threshold for serum zonulin concentration at 11-14. weeks of pregnancy was >36.783 (ng/mL) for predicting GDM. We suggest that serum zonulin levels should be used as a screening tool in the prenatal period or early pregnancy for biochemical determination of the association between serum zonulin levels and GDM. In addition to assessing zonulin levels, the incorporation of various biochemical parameters, such as insulin, C-reactive protein, high-density lipoprotein, low-density lipoprotein, cholesterol, interleukin-1, and IL-6, can also prove beneficial in the diagnostic process. In addition, it is recommended that larger cohorts of patients be included in future studies. However, in the postpartum period, patients with GDM should undergo a 75-g OGTT test within the first 6 months. Close follow-up and thorough observation are crucial to avoid short- and long-term complications associated with GDM. We also recommend that patients be carefully monitored simultaneously by a registered dietitian and an internal medicine specialist.

ETHICS

Ethics Committee Approval: This study was conducted with the approval of the Ethics Committee for Noninterventional Research of Wma Declaration Of Helsinki-Ethical Principles Formedical Research Involving Human Subjects (date: 20.08.2020, decision number: 626).

Informed Consent: Following the distribution of the informed consent form to all participants, the purpose of the study was shared with them to secure their voluntary participation in the research.

FOOTNOTES

Authorship Contributions

Surgical and Medical Practices: C.C., İ.A.Ö., Concept: C.C., L.H.A.T., V.Ü., İ.A.Ö., Design: C.C., L.H.A.T., V.Ü., İ.A.Ö., Data Collection or Processing: C.C., Analysis or Interpretation: C.C., M.C., Literature Search: C.C., M.C., Writing: C.C., M.C., İ.A.Ö.

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

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