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MATERNAL-FETAL MEDICINE

Prediction of gestational diabetes mellitus and perinatal outcomes by plasma zonulin levels

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Abstract

Purpose Zonulin has been shown to be associated with many metabolic disorders, including type 2 diabetes mellitus, metabolic syndrome, and obesity. In this study, we aimed to evaluate the association between maternal plasma zonulin levels and gestational diabetes mellitus (GDM) and its perinatal outcomes.

Materials A total of 100 pregnant women, 56 with GDM and 44 controls, were included in this prospective case–control study. Maternal plasma zonulin levels were evaluated in each trimester. The association between zonulin levels and GDM, body mass index (BMI) and adverse perinatal outcomes was evaluated. The GDM predictability of zonulin levels for each trimester was analyzed with the receiver operator curve (ROC).

Results Plasma zonulin levels were significantly higher in pregnant with GDM in all trimesters ($p < 0.001$; for all). Optimum cut-off values of plasma zonulin levels in predicting GDM: first trimester: 6.27 ng/mL, second trimester: 12.71 ng/mL, and third trimester: 18.38 ng/mL. BMI was significantly higher in pregnant women with GDM (30.5 vs 26.1; $p < 0.001$). Zonulin levels were significantly higher in pregnant women with GDM with overweight BMI [≥ 25–30 (kg/m^2)] in all trimesters ($p < 0.05$; for all). Zonulin levels were significantly higher in pregnant women with composite adverse outcomes that included at least one of neonatal intensive care unit (NICU) admission, meconium-stained amniotic fluid, and 1st minute APGAR score < 7.

Conclusion Increased maternal plasma zonulin levels were associated with increased risk of GDM and adverse perinatal outcomes. Zonulin may be a potential marker to predict GDM risk and perinatal outcomes.

Keywords Zonulin · Gestational diabetes mellitus · Intestinal permeability

What does this study add to the clinical work

Plasma zonulin levels in pregnants with GDM were higher for each trimester than controls and could predict the risk of GDM. In addition, increased plasma zonulin levels in pregnants with GDM were associated with adverse perinatal outcomes.

Introduction

Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance that begins or is detected for the first time during pregnancy. The prevalence of GDM ranges from 9 to 26% (mean 18%), depending on the population characteristics and the diagnostic criteria used [1]. The prevalence is

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expected to increase further in the future due to the increase in advanced maternal age, obesity and sedentary life [2]. GDM has a complex etiology that includes genetic and environmental factors. It could be pregestational insulin resistance unmasked during pregnancy, but not always, it could be a risk factor for future developing of type 2 diabetes [3]. It is associated with some adverse perinatal outcomes for the mother and fetus such as macrosomia, preterm delivery, birth injury, neonatal hypoglycemia, neonatal intensive care unit (NICU) admission, respiratory distress, gestational hypertension, preeclampsia, and cesarean section [4, 5]. Studies have also shown that growing up in a long-term intrauterine diabetic environment can have long-term effects, such as developing obesity, type 2 diabetes, metabolic syndrome, and cardiovascular disease in adulthood [6–8].

Recent studies have shown that increased intestinal permeability is associated with several disorders associated with low-grade inflammation, including obesity, obesity-related insulin resistance, and type 2 diabetes [9, 10]. Impaired gut microbiota has also been shown to be involved in the pathogenesis of inflammatory bowel disease, autoimmune diseases, diabetes mellitus, obesity, coronary artery disease, and colon cancer [11]. The mechanism of formation of these diseases is stated as the circulating of the lipopolysaccharides of the bacteria due to impaired intestinal permeability and impaired gut microbiota and causing metabolic endotoxemia, leading to low-level inflammation [12, 13]. Zonulin is the essential protein that modulates tight junctions to regulate intercellular transport [14]. It works as an endogenous regulator of intestinal paracellular permeability disassembling tight junctions [15]. Recent studies have shown that there is an increase in maternal plasma and serum zonulin levels in GDM, preeclampsia and intrahepatic cholestasis of pregnancy [16, 17].

Despite the few animal and human studies showing the relationship between zonulin and diabetes, there is not enough data on zonulin levels in pregnancy and GDM, especially in the early and later stages of pregnancy. In this study, we aimed to evaluate maternal plasma zonulin levels and changes in pregnant women with GDM in each trimester, as well as the association of zonulin levels with maternal characteristics and perinatal outcomes.

Materials and methods

This prospective case–control study was conducted between January 2020 and December 2021 at University of Health Sciences Samsun Training and Research Hospital, Department of Obstetrics and Gynecology. The study protocol was approved by the local institutional Ethics Committee (approval number: OMU KAEK 2021/97). Informed consent was obtained from all participants.

Pregnant women above 18 years with or without GDM were included in the study (cases and controls, respectively). The definition of GDM has been established according to the current guidelines of the International Association of Diabetes in Pregnancy Study Groups (IADPSG) [18]. 75 g oral glucose tolerance test (OGTT) was performed on all pregnant women between 24 and 28 weeks of gestation. Fasting plasma glucose value ≥ 92 mg/dl (5.1 mmol/L) and/or 1-h glucose value ≥ 180 mg/dl (10.0 mmol/l) and/or 2-h glucose value ≥ 153 mg If/dl (8.5 mmol/l) included at least one parameter from these measurements, pregnant women were defined as GDM.

Inclusion criteria for the study: (1) single pregnancy; (2) 75-g OGTT performed between 24 and 28 weeks of gestation; (3) plasma zonulin levels measured in each trimester. Exclusion criteria were (1) multiple pregnancies (2) pregnant woman with pregestational diabetes mellitus, (3) pregnant women with additional diseases (autoimmune disorders, liver or kidney diseases, active infection, thyroid dysfunction, and intestinal diseases).

Plasma zonulin levels of all participants were measured in each trimester; between 11 and 14 weeks of gestation in the first trimester, between 24 and 28 weeks of gestation in the second trimester, and between 36 and 40 weeks of gestation in the third trimester. For zonulin measurement, blood samples were taken by venipuncture and processed by centrifugation at 5000 rpm for 15 min within 1 h after collection, and all plasma samples were stored at – 80 °C until the day of analysis. To provide an adequate case and control group, first trimester plasma zonulin levels of pregnant women with GDM were measured retrospectively from maternal blood samples collected in the first trimester and stored at – 80 °C. Plasma Human Zonulin concentrations were measured using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits (Sun-Red Bio Company, Cat No. 201-12-5578, Shanghai, China) on the Cobas C501 Analyzer (Roche Diagnostics, Rotkreuz, Switzerland) in the Biochemistry Laboratory of Samsun Training and Research Hospital. This kit uses a double antibody sandwich enzyme-linked immunosorbent assay to test the Human Zonulin level. Enzymatic reactions were measured in an automated microplate photometer. Human zonulin levels were expressed as ng/mL. The sensitivity of this test is 0.223 ng/mL and the test range is between 0.25 and 70 ng/mL. All tests were performed according to the manufacturer's instructions. Samples showing higher concentrations were diluted and measured in duplicate.

Maternal characteristics such as age, parity, and body mass index (BMI) of each pregnant woman participating in

the study were recorded. Birth weeks, delivery types, birth weights and neonatal outcomes were collected from the hospital digital record system.

Statistical analysis

Data were analyzed with Statistical Package for the Social Sciences (SPSS) v26.0 (IBM® SPSS® Statistics, New York, USA). For the analysis of the data, first of all, the normality distribution was examined. The normality of the distributions was evaluated with the Shapiro–Wilk test. Student's *t* test was used for normally distributed data and results were presented as mean ± SD. The Mann–Whitney *U* test was used for the data not normally distributed and the data were shown as median (min, max). Categorical variables were compared with the chi-square test. The area under the receiver operating characteristic (ROC) curve was used for cut-off values, sensitivity, and specificity. $p < 0.05$ were considered significant.

Results

A total of 100 pregnant women, 56 with GDM and 44 controls, were included in the study. Maternal characteristics and perinatal outcomes of pregnant women are presented in Table 1. There were no significant differences between maternal age and gravidity of pregnant women with GDM and controls. BMI was significantly higher in pregnant women with GDM (30.5 (23.1–38.1) vs 26.1 (22.1–42.1); $p < 0.001$). Gestational age at delivery was similar in both groups; however, mean birth weight and macrosomia rate were significantly higher in diabetic pregnancies ($p = 0.002$, $p = 0.017$; respectively). Cesarean delivery rate was significantly higher in diabetic pregnancies (24 (43%) vs 7 (16%); $p = 0.004$). Adverse perinatal outcomes were significantly higher in pregnant women with GDM, and at 1st- and 5th-minute APGAR scores were significantly lower in pregnant women with GDM (Table 1).

Maternal plasma zonulin levels in each trimester of both groups are presented in Table 2. Plasma zonulin levels were significantly higher in pregnant women with GDM in all trimesters compared to controls ($p < 0.001$; for all) (Fig. 1). We also found that there was a significant increase in plasma zonulin levels in the GDM group during pregnancy period ($p < 0.05$; for all) (Table 2). In the control group, second trimester and third trimester plasma zonulin levels were significantly higher than first trimester zonulin levels ($p < 0.001$, $p = 0.012$; respectively); however, there was no significant difference in zonulin levels between the second and third trimesters ($p = 0.165$) (Table 3). Optimum cut-off values and diagnostic data of plasma zonulin levels in predicting GDM: first trimester cut-off: 6.27 ng/mL, sensitivity: 76.8%, specificity: 75%, AUC: 0.790, 95% CI 0.697–0.882, second trimester cut-off: 12.71 ng/mL, sensitivity: 72.7%, specificity: 71.4%, AUC: 0.796, 95% CI 0.710–0.881, and third trimester cut-off: 18.38 ng/mL, sensitivity: 75.4%, specificity: 64.9%, AUC: 0.799, 95% CI 0.704–0.894 ($p < 0.001$; for all). (Fig. 2).

Table 1 Maternal characteristics and perinatal outcomes of the pregnant woman included in the study

	Gestational diabetes $n = 56$	Control $n = 44$	p
Maternal age (year) median (min–max)	26 (19–40)	25 (20–33)	0.082
BMI at during test (kg/m^2) median (min–max)	30.5 (23.1–38.1)	26.1 (22.1–42.1)	< 0.001
Gravidity median (min–max)	2 (1–5)	2 (1–5)	0.725
Birth weight (gram) (mean ± SD)	3929 ± 743	3504 ± 580	0.002
Gestational age at delivery (week) (mean ± SD)	39 ± 1	39 ± 2	0.943
Cesarean section (*n*, %)	24 (43%)	7 (16%)	0.004
Macrosomia (≥ 4000 g) (*n*, %)	21 (37.5%)	7 (16%)	0.017
NICU admission (*n*, %)	13 (23%)	2 (5%)	0.009
Meconium-stained amniotic fluid (*n*, %)	9 (16%)	2 (5%)	0.069
APGAR Score at 1st minute median (min–max)	7.95 (5–9)	8.86 (5–9)	< 0.001
APGAR Score at 5th minute median (min–max)	9.41 (7–10)	9.93 (8–10)	0.003

BMI body mass index, *NICU* neonatal intensive care unit

Table 2 Comparison of maternal zonulin plasma levels for each trimester between GDM and the control group

	Gestational diabetes $n = 56$	Control $n = 44$	p
1st trimester (ng/mL) median (min–max)	12.6 (0.67–46.64)	3.76 (0.45–44.12)	< 0.001
2nd trimester (ng/mL) median (min–max)	29.3 (3–89)	7.5 (0.7–44.6)	< 0.001
3th trimester (ng/mL) median (min–max)	43.36 (0.45–103.1)	5.2 (0.16–101)	< 0.001

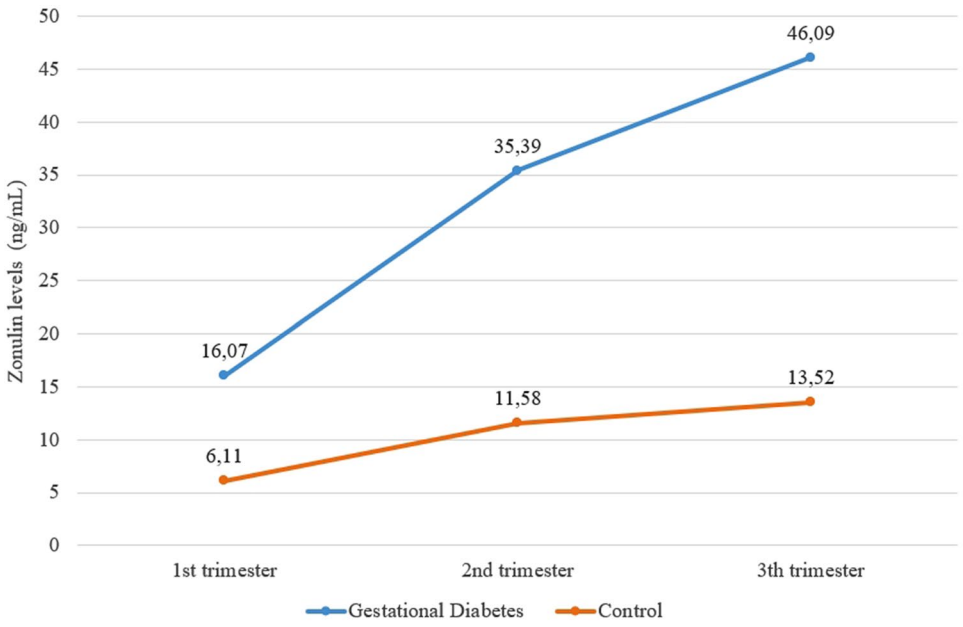

Fig. 1 Plasma mean zonulin levels according to trimesters in GDM and control groups

Table 3 Comparison of maternal zonulin plasma levels for each trimester

	Gestational diabetes	Control
1st trimester vs 2nd trimester	< 0.001	< 0.001
1st trimester vs 3th trimester	< 0.001	0.012
2nd trimester vs 3th trimester	0.042	0.165

The examination of maternal plasma zonulin levels according to BMI levels is presented in Table 4. Maternal plasma zonulin levels were significantly higher in pregnant women with GDM at all trimesters, in the overweight range of BMI [≥ 25–30 (kg/m^2)] ($p = 0.002$, $p = 0.005$, $p < 0.001$, respectively). However, there was no significant difference between maternal plasma zonulin levels in all trimesters

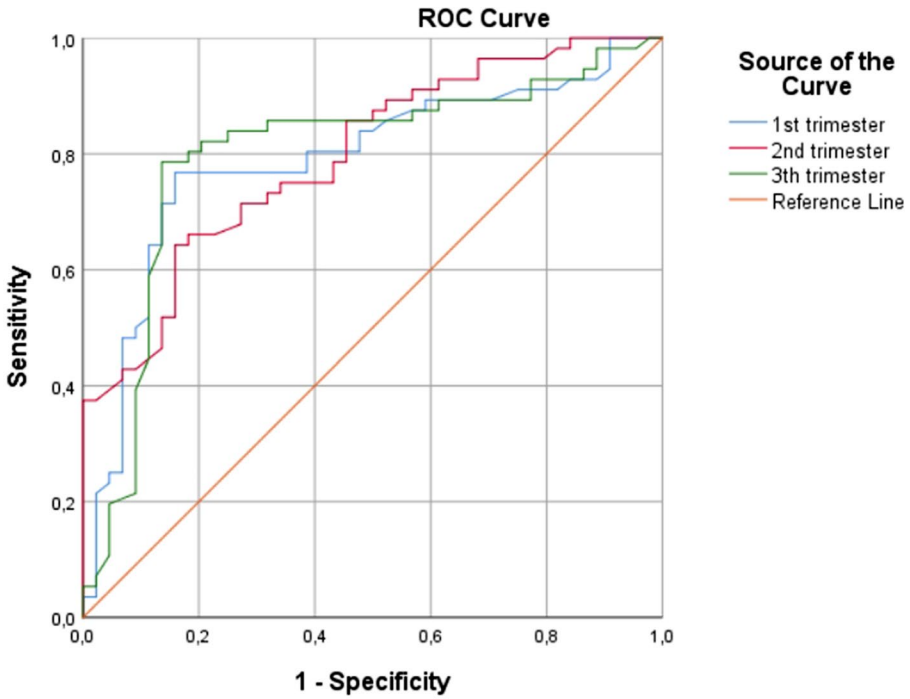

Fig. 2 Optimum cut-off values and diagnostic data of plasma zonulin levels to predict GDM in the first, second and third trimesters

Table 4 Examination of zonulin levels according to body mass index levels

	Gestational diabetes	Control	*p*
BMI at during test healthy weight range ≥ 18.5–25 (kg/m^2) *n* = 42			
1st trimester (ng/mL) median (min–max)	2.56 (0.27–8.17)	3.86 (1.01–6.23)	0.640
2nd trimester (ng/mL) median (min–max)	4.14 (0.66–10.26)	11.33 (4.88–12.66)	0.176
3th trimester (ng/mL) median (min–max)	4.97 (0.81–19.71)	5.19 (3.70–6.81)	0.665
BMI at during test overweight range ≥ 25–30 (kg/m^2) *n* = 32			
1st trimester (ng/mL) median (min–max)	10.65 (0.88–46.64)	3.45 (0.56–9.12)	0.002
2nd trimester (ng/mL) median (min–max)	12.77 (4.99–88.99)	5.12 (0.66–23.88)	0.005
3th trimester (ng/mL) median (min–max)	27.08 (0.45–101.20)	4.56 (0.16–9.95)	< 0.001
BMI at during test obesity ≥ 30 (kg/m^2) *n* = 26			
1st trimester (ng/mL) median (min–max)	17.88 (0.67–44.69)	13 (0.45–44.12)	0.615
2nd trimester (ng/mL) median (min–max)	53.76 (2.99–88.75)	33.95 (0.67–44.55)	0.085
3th trimester (ng/mL) median (min–max)	67.44 (0.77–103.11)	66.62 (0.56–101.11)	0.858

BMI body mass index

Table 5 Evaluation of adverse perinatal outcomes according to maternal zonulin plasma levels for the third trimester in gestational diabetes and control groups

	Gestational diabetes group *n* = 56 Zonulin Levels (ng/mL) (mean ± SD)	*p*		Control group *n* = 44 Zonulin Levels (ng/mL) (mean ± SD)	*p*
NICU admission		0.116	NICU admission		N/A
Yes (*n* = 13)	58.70 ± 32.34		Yes (*n* = 2)	4.45 ± 1.05	
No (*n* = 33)	42.27 ± 32.52		No (*n* = 42)	13.96 ± 25.83	
Meconium-stained amniotic fluid		0.005	Meconium-stained amniotic fluid		N/A
Yes (*n* = 9)	73.74 ± 27.01		Yes (*n* = 2)	4.45 ± 1.05	
No (*n* = 47)	40.79 ± 31.49		No (*n* = 42)	13.96 ± 25.83	
1st minute APGAR scores		0.003	1st minute APGAR scores		N/A
< 7 (*n* = 8)	77.21 ± 21.13		< 7 (*n* = 1)	13.75 ± 25.56	
≥ 7 (*n* = 48)	40.90 ± 31.79		≥ 7 (*n* = 43)	3.70	
5th minute APGAR scores		N/A	5th minute APGAR scores		N/A
< 7 (*n* = 0)	–		< 7 (*n* = 0)	–	
≥ 7 (*n* = 56)	46.09 ± 32.94		≥ 7 (*n* = 44)	13.52 ± 25.30	
Composite adverse outcomes		0.005	Composite adverse outcomes		N/A
Yes (*n* = 18)	63.68 ± 31.26		Yes (*n* = 2)	4.45 ± 1.05	
No (*n* = 38)	37.75 ± 30.69		No (*n* = 42)	13.96 ± 25.83	

NICU Neonatal Intensive Care Unit

between pregnant women with GDM and controls with BMI value in the normal range [≥ 18.5–25 (kg/m^2)] and obesity level [≥ 30 (kg/m^2)].

The association between third trimester maternal plasma zonulin levels and adverse perinatal outcomes in pregnant women with GDM and control group is presented in Table 5. Statistical analysis could not be made due to the low number of adverse perinatal outcomes in the control group. In pregnant women with GDM, there was no significant difference between maternal plasma zonulin levels in pregnancies with and without NICU admission ($p = 0.116$). However, zonulin levels were significantly higher in pregnancies with meconium-stained amniotic fluid and 1st minute APGAR score < 7 ($p = 0.005$, $p = 0.003$, respectively). Zonulin levels were significantly higher in pregnant women with composite adverse outcomes that included at least one of NICU admission, meconium-stained amniotic fluid, and 1st minute APGAR score < 7 ($p = 0.005$).

Discussion

In this study, we evaluated maternal plasma zonulin levels in pregnant women with GDM and controls. Our data showed that plasma zonulin levels were significantly higher in pregnant women with GDM at each trimester, and plasma zonulin levels could predict GDM risk. When all pregnant women were grouped according to BMI, zonulin levels in the overweight BMI range [≥ 25–30 (kg/m^2)] remained significantly higher in pregnant women with GDM. In addition, there was a significant association between third trimester zonulin levels and adverse perinatal outcomes in diabetic pregnancies.

Gut microbiota refers to the whole of the microorganisms present in the digestive system. It has an important role in immune system development, metabolism of nutrients, protection from pathogens, and the incidence of many chronic diseases [19, 20]. Studies have shown that impaired normal gut microbiota composition is associated with type 2 diabetes mellitus, metabolic syndrome, and inflammatory bowel disease [21–23]. In an animal study, Koren et al. observed that changes occur in the intestinal microbiota in normal pregnancy, similar to inflammatory bowel disease and obesity. They also showed that when the mice were colonized with the third trimester maternal gut microbiota, elevated inflammatory responses, and insulin insensitivity occurred in the mice [24]. In addiction, Crusell et al. showed that GDM is associated with changes in the gut microbiota of third trimester and postpartum pregnant women [25]. Zonulin is located in the intestinal epithelium and acts as a physiological modulator of tight junctions. Several mechanisms, such as the alteration of the gut microbiota, may lead to a decrease in these connections and an increase in intestinal permeability [26]. Plasma zonulin levels have been used as a potential marker of intestinal permeability [9].

In recent studies, it has been suggested that an increase in plasma zonulin levels may occur due to increased intestinal permeability in pregnant women with GDM [16, 27, 28]. First, Mokkala et al. showed that first trimester maternal serum zonulin levels were significantly higher in pregnant women complicated with GDM in the mid-trimester. They also found that GDM could be predicted by first trimester zonulin levels [28]. Demir et al. evaluated maternal plasma zonulin levels between 24 and 28 weeks of gestation and found that second trimester plasma zonulin levels were significantly higher in pregnant women with GDM. Similar to Mokkala et al. they showed that zonulin levels in the second trimester could predict GDM [27]. Guvey et al. evaluated the plasma zonulin levels in intrahepatic cholestasis of pregnancy (ICP) and GDM cases together and found that zonulin levels were significantly higher in both ICP and GDM cases compared to controls. They also found that zonulin levels were highest in pregnancies complicated by both ICP and GDM [16]. In our study, we examined the changes in maternal plasma levels in pregnant women with GDM and control group in each trimester. We found a significant increase in plasma zonulin levels during pregnancy period in pregnant women with GDM. In addition, second and third trimester zonulin levels were significantly higher than first trimester zonulin levels in the control group. Metabolic changes in response to high fetal glucose demands during pregnancy and increased placental and local hormones in response may be associated with changes in plasma zonulin levels between trimesters [29]. Plasma zonulin levels of pregnant women with GDM were significantly higher than controls in all trimesters. We also found that zonulin levels in each trimester could predict GDM risk. The optimal zonulin cut-off value, sensitivity and specificity that can predict GDM differ between studies. Mokkala et al. evaluated serum zonulin levels of 88 pregnant women in the first trimester. The cut-off value was 43.3 ng/mL with 88% sensitivity and 47% specificity [28]. Bawah et al. also evaluated first trimester serum zonulin levels with 314 participants and the cut-off value was 47.5 ng/mL with 80.95% sensitivity and 80.41% specificity [30]. Demir et al. evaluated second trimester plasma zonulin levels with 175 participants and found the cut-off value of 20 ng/mL with 98.8% sensitivity and 100% specificity [27]. In our study, optimum cut-off values of plasma zonulin levels in predicting GDM: first trimester cut-off value: 6.27 ng/mL, sensitivity: 76.8%, specificity: 75%, second trimester cut-off value: 12.71 ng/mL, sensitivity: 72.7%, specificity: 71.4%, and third trimester cut-off value: 18.38 ng/mL, sensitivity: 75.4%, specificity: 64.9%. We thought that these differences might be due to differences in gestational weeks, maternal characteristics, geographical nutritional characteristics and the number of participants. Large-scale and multicenter studies are required for optimal zonulin cut-off value in GDM predictability.

The association between maternal BMI levels and intestinal microbiota and zonulin levels has been demonstrated in previous studies [31, 32]. Many studies have also shown that high BMI levels cause an increased risk of GDM [31, 32]. Therefore, differences in BMI levels may cause confounding results, as both GDM and zonulin levels are expected to be high in cases with high BMI. Mokkala et al. selected both control and GDM participants with similar BMI levels in order to exclude the effect of BMI level on zonulin levels in their study. However, their studies included a small number of GDM cases and provided results only on zonulin levels of pregnant women with high BMI levels (mean 30.8 kg/m^2) [28]. In our study, BMI levels in GDM cases were significantly higher than in controls. To exclude the effect of BMI levels on zonulin levels, we classified GDM cases according to their BMI levels. We found that zonulin levels in pregnant women with normal and obesity BMI ranges were similar to the control groups. However, zonulin levels

were significantly higher in cases with GDM in the overweight BMI range compared to controls. Guvey et al. also grouped the pregnant women according to their BMI levels and showed that the zonulin levels of the GDM cases were significantly higher than the controls in the overweight and obesity ranges, but similar to our study, no significant difference was found between the zonulin levels in both groups within the normal BMI range [16]. This difference may be due to the inclusion of ICP cases in the study of Guvey et al. or the differences in the number and characteristics of the participants.

It is well known that GDM causes adverse maternal and fetal perinatal outcomes. [4, 5]. However, there is no generally accepted model predicting the risk of adverse perinatal outcomes. In this study, our data showed that adverse perinatal outcomes were significantly higher in pregnant women with GDM, and at 1st- and 5th-minute APGAR scores were significantly lower in pregnant women with GDM. It also showed that zonulin levels were significantly higher in diabetic pregnant women with meconium-stained amniotic fluid, 1st minute APGAR score < 7, and composite adverse perinatal outcomes. Similarly, Guvey et al. showed that there was a positive and significant correlation between zonulin levels and adverse perinatal outcomes such as NICU admission, low APGAR scores at 1st and 5th minutes, meconium-stained amniotic fluid [16]. According to these results, zonulin might be a potential marker that could predict adverse perinatal outcomes in GDM; however, further studies with large participation are needed.

The main limitation of this study is the small number of participants since it was designed prospectively in a single center. Evaluation of the association between BMI levels and zonulin levels was only possible with a small number of participants. In addition, due to the lack of laboratory values such as glucose, insulin, HbA1c, and, HOMA-IR, the association between glucose regulation and plasma zonulin levels could not be evaluated. The important strength of this study is the evaluation of zonulin levels in all cases in each trimester.

In conclusion, maternal plasma zonulin levels were higher for each trimester in pregnant women with GDM, and plasma zonulin levels could predict the risk of GDM. In addition, increased plasma zonulin levels in patients with GDM were associated with adverse perinatal outcomes. Zonulin, which is associated with gut microbiota and permeability, may be a potential marker to predict GDM risk and adverse perinatal outcomes, however, these findings should be supported by large population-based studies.

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Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

Ethical approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Ondokuz Mayıs University (approval number: OMU KAEK 2021/97).

Consent to participate Informed consent was obtained from all individual participants included in the study.

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