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Zonulin: can it be used as a marker for preterm labor?

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Abstract. – OBJECTIVE: Inflammation has been reported to increase zonulin levels, a protein that regulates intestinal permeability. The aim of this study was to investigate the association of serum zonulin levels with preterm labor in pregnant women.

PATIENTS AND METHODS: A total of 180 pregnant women between 32-42 weeks of gestation were included in the study. Among those whose gestational week is less than 37, preterm labor (group 1), normal course pregnant (group 2), and gestational weeks between 37-42 pregnant women with normal course (group 3), term labor (group 4) groups were formed.

RESULTS: Zonulin levels were not statistically different between groups. Among the inflammation markers, only C-reactive protein levels were significantly higher in group 1 compared to groups 2 and 3. There were a total of 18/90 (20%) pregnant women with premature rupture of membranes (PROM) in the delivery groups. The mean zonulin level was higher in those with PROM (155.3±50.2 ng/ml) than those without PROM (128.8±59 ng/ml). However, there was no statistically significant difference between them.

CONCLUSIONS: In conclusion, serum zonulin levels were not useful as a marker for predicting preterm labor. However, examining large-scale studies about the relationship between PROM and zonulin is still needed.

Key Words:
Preterm labor, Pregnancy, Inflammation, Zonulin.

Introduction

Zonulin is a protein released from hepatocytes, enterocytes, adipose tissue and immune cells, and regulates tight junctions between intestinal epithelial cells[1,2]. Stimulation of zonulin release has been shown to result in detachment of zonula occludens-1 protein from tight junctions, which results in increased intestinal permeability[3]. Therefore, zonulin increase has been used to indicate intestinal permeability change.

Zonulin is a part of the innate immune system of the gut. It is upregulated in severe autoimmune diseases, such as celiac disease and type 1 diabetes mellitus[4-6]. Studies[7-11] have reported increased zonulin levels in conditions including sepsis, gestational hypertension, gestational diabetes mellitus, intrahepatic cholestasis of pregnancy, and polycystic ovary syndrome. In a study conducted on obese patients, a positive correlation was reported between zonulin levels and IL-6, which is a marker of inflammation[12].

One of the most important causes of preterm deliveries is inflammation caused by infection[13]. In addition, sterile inflammation caused by non-infectious origins has also been reported to lead to preterm delivery[14]. To determine the risk of preterm delivery, measurement of inflammation markers, such as IL-6 from amniocentesis in pregnant women or IL-6 rapid test in vaginal fluid in the presence of premature rupture of membranes (PROM), can be performed[15,16]. However, these are not routinely used. In addition, amniocentesis, which is a highly invasive procedure, alone poses a risk of preterm delivery.

The aim of the study was to investigate whether serum zonulin levels are associated with preterm labor in pregnant women and whether it can be used as a marker to predict preterm labor. The secondary objective of this study was to examine the relationship between zonulin levels and conditions that may increase inflammation, such as PROM and low Apgar score.

Patients and Methods

The study was conducted prospectively in Erzurum Regional Training and Research Hospital between July 2022 and June 2023. This hospital is a reference center where approximately 10 thousand births occur annually. Approval for the study was obtained from the Ethics Committee of

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Erzurum Regional Training and Research Hospital and registered (registration number: 2022/07-64). The informed consent form was obtained from the patients for the study before enrollment.

Inclusion Criteria

Women aged 18-35 years with a gestational age of 32 weeks or more and a singleton pregnancy were included in the study. Only pregnant women who delivered vaginally were included in the study.

Exclusion Criteria

Women with chronic diseases and continuous medication, any bowel disease and gastrointestinal tract surgery, smoking, uterine anomalies, any complications and risk factors related to pregnancy (hypertension, diabetes mellitus, pre-eclampsia/eclampsia, HELLP, cholestasis, intrauterine growth restriction) were excluded.

Patient Groups and Data Collection

Group 1: Pregnant women who started preterm labor between 32 and 36^6 weeks.
Group 2: Pregnant women between 32-36^6 weeks of gestation who did not go into labor.
Group 3: Pregnant women between 37 and 42 weeks who did not go into labor.
Group 4: Pregnant women in labor between 37 and 42 weeks.

Parity, gravidity, age, presence of PROM, body weight, Apgar scores, and neonatal intensive care unit (NICU) requirement data of pregnant women were included and recorded on the study form. Routine hemogram, procalcitonin, C-reactive protein (CRP), and procalcitonin levels were obtained simultaneously with the samples taken for zonulin and analyzed immediately with the appropriate method in the hospital laboratory.

Study of Serum Zonulin Levels

Venous blood samples were collected from group 1 and group 4, who had preterm labor and normal term labor at the 1st stage of labor, and from group 2 and group 3, who had a normal course of pregnancy without the onset of labor, at the outpatient clinic control visits.

The samples were placed in anticoagulant-free tubes and kept at room temperature for 20 minutes for clotting. Then, they were centrifuged at 3,000 rpm for 20 minutes in accordance with the Enzyme-Linked Immunosorbent Assay (ELISA) kit instructions. The upper serum portions were transferred to low protein binding microtubes (Eppendorf, Germany) and stored at -80 degrees Celsius until the date of the study. Zonulin analysis was performed by ELISA method using the Human Zonulin ELISA kit (Bioassay Technology Laboratory, cat no: E3704HU, Shanghai, China).

Statistical Analysis

Statistical analysis was performed using SPSS 23 software (IBM Corp., Armonk, NY, USA). Shapiro-Wilk and Kolmogorow-Smirnov tests were used to analyze normality distribution. It was determined that all data in the study group had a parametric distribution. Independent samples *t*-test and ANOVA test were used to compare continuous data between groups. Post-hoc analysis was performed using the Bonferroni test for significant results in the ANOVA test. Chi-square tests were used to compare categorical data. Pearson correlation tests were used to analyze whether the continuous variables were related. A two-tailed p lower than 0.05 was accepted as significant.

Results

A total of 180 pregnant women, 45 in each group, were included in the current study. When demographic data were compared between the groups, the mean maternal age was group 1: 28.7±6; group 2: 30.4±5.4; group 3: 29.7±4.7; and group 4: 28.1±6.5, respectively. There was no statistically significant difference between the groups for maternal age (p=0.12). There was no statistically significant difference between the groups for the weight of the pregnant women (p= 0.09). Gravity and parity numbers were not different between the groups (p=0.284, p=0.396, respectively). Gestational ages were statistically different between the groups (Table I).

Inflammation markers [white blood cell count (WBC), CRP, procalcitonin] and zonulin levels were compared between the groups. There was no statistically significant difference between the groups in zonulin, WBC, and procalcitonin levels (p=0.83, p=0.09, p=0.385, respectively). However, there was a statistically significant difference in the mean CRP levels between the groups (p=0.02) (Table II). Post-hoc analysis revealed that the mean CRP level in group 1 was statistically and significantly higher than group 2

Table I. Comparison of demographic data of pregnant women between groups.

Features	Group 1* (n = 45)	Group 2* (n = 45)	Group 3* (n = 45)	Group 4* (n = 45)	p**
Age (year)	28.7 ± 6.0	30.4 ± 5.4	29.7 ± 4.7	28.1 ± 6.5	0.12
Body weight (kg)	71.5 ± 12.5	76.4 ± 12.5	76.6 ± 11.7	72.4 ± 10.8	0.09
Gestational age (week)	34.1 ± 2.1	32 ± 2.4	37.7 ± 0.8	38.9 ± 1.2	< 0.01
Gravity (n)	2.7 ± 1.5	2.8 ± 1.5	2.3 ± 1.0	2.6 ± 1.8	0.284
Parity (n)	1.4 ± 1.5	1.5 ± 1.3	1.1 ± 0.9	1.4 ± 1.6	0.396

*Presented as mean ± standard deviation. **A comparison of groups was performed using the ANOVA test. $p < 0.05$ was considered significant.

Table II. Comparison of inflammatory markers between groups.

Marker	Group 1* (n = 45)	Group 2* (n = 45)	Group 3* (n = 45)	Group 4* (n = 45)	p**
Zonulin (ng/ml)	134 ± 63.2	133.3 ± 81.9	123.6 ± 56.7	134.1 ± 53.1	0.83
Procalcitonin (ng/ml)	0.13 ± 0.42	0.05 ± 0.07	0.07 ± 0.05	0.07 ± 0.07	0.38
WBC (10^3/µl)	13.3 ± 5.7	10.3 ± 1.9	9.07 ± 2.5	11.9 ± 1.5	0.09
CRP (mg/dl)	19.2 ± 15.3	6.35 ± 5.72	6.98 ± 6.6	12.5 ± 9.3	0.002

*Presented as mean ±s tandard deviation. **A comparison of groups was performed using the ANOVA test. $p < 0.05$ was considered significant.

and group 3 (p=0.004, p=0.009, respectively). In addition, the group 1 mean CRP level was 19.2±15.3 mg/dl, which was higher than the group 4 mean CRP value of 12.5±9.3 mg/dl. However, there was no statistically significant difference between them (p=0.14). There was also no significant difference between group 4 CRP levels and group 2 and group 3 CRP levels (p=0.14, p=0.26, respectively).

The mean serum zonulin value in the study group was 131.2±64.3 ng/ml. The number of PROM was 18 (20 %) in pregnant women who gave birth (group 1 and group 4, n=90). The mean zonulin levels were higher in those with PROM (155.3±50.2 ng/ml) than those without EMR (128.8±59 ng/ml). However, there was no statistically significant difference between them (p=0.063). There was also no significant difference in the incidence of EMR between group 1 and group 4 (p=0.39). There was no correlation between zonulin levels and 1st, and 5th minute Apgar scores of newborn babies (p=0.77, p=0.91, respectively). There was no correlation between birth weight and zonulin levels (p=0.52). Of 90 babies, 22 (24.4 %) needed NICU. There was no significant difference in zonulin levels between mothers whose babies were hospitalized in NICU and those whose babies were not (p=0.36) (Table III).

Discussion

The most important cause of the preterm labor is the perinatal infection. Apart from bacterial infections and chorioamnionitis, the COVID-19 and Zika viruses, which have recently come to the fore, can also cause premature birth[17,18]. In a recent research study examining pregnant women diagnosed with COVID-19, it was reported that there was a 20% rate of preterm birth[17]. The Zika

Table III. Evaluation of the relationship between premature rupture of membranes, Apgar scores, birth weight of newborns, neonatal intensive care unit requirement, and zonulin levels.

Parameter		p**
PROM*	Present (n = 18) Absent (n = 72)	0.063**
1st minute apgar	0.77***	
5th minute apgar	0.91***	
NICU*	Present (n = 22) Absent (n = 68)	0.36**
Birth weight		0.52***

*PROM: premature rupture of membranes; NICU: neonatal intensive care unit. **Statistics were performed using independent samples t-test. $p < 0.05$ was accepted as significant. *** Statistics were performed using the Pearson correlation test. $p < 0.05$ was accepted as significant.

virus infection is a cause that can lead to fetal anomaly in the fetus, particularly by affecting the central nervous system[18]. In this study, there were no pregnant women who had intrauterine fetal malformation. In addition, pregnant women with risks, such as intrauterine growth restriction, were not included in the study. However, it is not possible to predict asymptomatic perinatal infection in the current study. In addition to the infections, pregnancy over the age of 35 is also an important risk factor for premature birth[19]. Relevant research in the literature reported a preterm birth rate of up to 59% in pregnant women over the age of 35[19]. Women aged 18-35 years with a gestational age of 32 weeks or more and with a singleton pregnancy were included in the study.

In this study, the women who had spontaneous vaginal delivery were included in the birth groups (group 1 and 4). Although waterbirth could be performed in the hospital, it was not preferred because of its neonatal complications[20]. It is known that in high-risk pregnancies, the entire metabolism is affected, and even serum sodium and potassium levels may become abnormal values[21]. Therefore, while designing the study, high-risk pregnancies that might affect zonulin levels were excluded.

In this study, zonulin levels in the preterm labor group were not statistically different from the other groups. This information demonstrates that zonulin is not useful in predicting preterm labor. Indeed, the hypothesis was formulated based on the understanding that maternal infection constitutes a significant factor in preterm labor[13]. It is known that exposure to pathogenic bacteria in the intestine leads to an increase in zonulin levels and, thus, rearrangement in tight junction proteins and increased intestinal permeability[4,22]. This has been reported to be an immune response of the intestine to prevent colonization of pathogens[4,23].

In the study conducted by Klaus et al[7], zonulin levels of 25 patients diagnosed with sepsis, 18 postoperative non-septic patients, and 20 healthy volunteers were compared. Zonulin levels were significantly higher in patients diagnosed with sepsis compared to the other two groups. In the current study, there were no patients with a proven diagnosis of sepsis. However, CRP levels were significantly higher in the study group with preterm labor in this study. It is known that histologic chorioamnionitis without proven or clinical sepsis is also a risk factor for preterm labor[24]. Perhaps elevated CRP levels may indicate such a condition. Examining the relationship between the presence of histologic chorioamnionitis without an evident infection or sepsis causing preterm delivery and zonulin levels may be the subject of a future study.

Increased intestinal permeability may not always result from a pathologic condition. Studies[25,26] have shown that exposure to gliadin in gluten increases intestinal permeability *via* zonulin. It has also been reported that zonulin increases in non-infectious conditions, such as obesity, diabetes mellitus, and gestational hypertension[9,10]. In this study, these diseases were excluded as they are often observed in pregnancy. Therefore, it is possible to affirm that intestinal permeability is not impaired in preterm labor patients when concomitant disease or proven sepsis is not included.

PROM increases the incidence of infection in pregnant women and newborns[27]. However, the risk of infection development can be reduced with prophylactic antibiotic treatment[28]. In this study, zonulin levels in pregnant women with PROM were higher than those without PROM.

Although there was no statistical difference between them, it is believed that this result holds value, indicating the necessity for studies with the primary objective of examining the relationship between PROM and zonulin.

To the best of current knowledge, no study has been published that investigated the relationship between maternal zonulin levels and Apgar score. Apgar score has been used to objectively evaluate the condition of the baby in the delivery room and has been shown to be affected by many maternal/neonatal factors; some of which are accompanied by inflammation[29]. In this study, there was no relationship between zonulin levels and Apgar scores.

Limitations

This study had some limitations. Since histologic chorioamnionitis was not evaluated, it was not possible to reach a clear conclusion about the relationship between infection and zonulin in mothers. Although pregnancy complications and maternal chronic diseases were excluded in the study, there may be other unpredictable parameters that may affect zonulin levels, such as dietary habits and gluten intake.

Conclusions

In conclusion, the measurement of serum zonulin levels does not seem to be useful in predicting preterm labor. However, considering the

relationship between premature rupture of membranes and infection, it is possible to affirm that elevated zonulin found in patients with PROM in this study is noteworthy. However, larger studies examining the relationship between zonulin and the causes of preterm labor are still needed.

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Conflict of Interest
The author declares no conflict of interest.

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Data Availability
The data supporting this article are available from the corresponding author upon reasonable request.

Ethics Approval
Approval was obtained from the Ethics Committee of Erzurum Regional Training and Research Hospital (approval number: 2022/07-64).

Informed Consent
The informed consent form was obtained from the patients involved in the study before enrollment.

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