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Is there an association between the serum zonulin concentration and the occurrence of PPROM?

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ABSTRACT

Aims: The aim is to evaluate the association between intestinal permeability, as assessed by zonulin levels, and preterm premature rupture of membranes (PPROM).

Methods: A prospective case-control study was conducted involving 44 pregnant women: 22 with PPROM and 22 matched controls. High-risk pregnancies (gestational diabetes, preeclampsia, multiple pregnancies), chronic diseases, and smoking were exclusion criteria. Demographic and clinical data were collected from medical records. Venous and umbilical cord blood samples were obtained post-delivery, centrifuged at 3000 rpm for 10 min, and stored at -80°C.

Results: No significant differences were found between the PPROM and control groups regarding age, body-mass index, gravidity, previous abortions, history of preterm rupture of membrane (PROM), or PPROM. Maternal and cord blood zonulin levels were comparable between groups ($p>0.05$). In the PPROM group, maternal and fetal cord zonulin levels correlated positively with newborn birthweight ($r=0.607$, $p=0.003$; $r=0.617$, $p=0.002$, respectively). A strong positive correlation was observed between maternal serum and fetal cord blood zonulin levels ($r=0.837$, $p<0.001$).

Conclusion: A positive correlation existed between newborn birthweight and both maternal and fetal cord zonulin levels in the PPROM group. Additionally, a strong positive correlation was observed between maternal serum and fetal cord blood zonulin levels in all participants.

Keywords: Zonulin, intestinal permeability, PPROM, premature preterm rupture of membrane, maternal serum, umbilical cord blood

INTRODUCTION

Zonulin is a 47 kDa molecular weight protein encoded by haptoglobin and acts to increase intestinal permeability. Zonulin was discovered identified by Wang et al.[1] through studies examining the pathophysiology of Vibrio cholerae, enabling the identification of zonulin as a secondary enterotoxin named the zonula occludens toxin (Zot) and its characterization as an endogenous homolog.[1] Fasano et al.[2] proposed that zonulin binds to the epidermal growth factor receptor (EGFR) and activates it. Zonulin can bind to protease-activated receptor type 2 (PAR2) and activate this receptor. When these receptors are activated, they initiate cell signaling, causing a reduction in protein interactions at tight junctions. As tight connections break down, antigens can freely move from the intestine to the surrounding tissue, leading to increased intestinal permeability.[2]

Although the exact role of zonulin in many diseases is not completely clear, increased levels of zonulin leading to increased loss of intestinal barrier function could trigger inflammation. This, in turn, may cause an imbalance or improper distribution of the microbiome throughout the gastrointestinal system. zonulin release can induce the crossing of the epithelial barrier, leading to the release of proinflammatory cytokines and causing microbiome imbalance.[3] The presence of cytokines continues to increase intestinal permeability, allowing for the significant transfer of nutrients and microbial antigens. This leads to the activation of T cells, which can migrate to the intestine or various organs, causing systemic chronic inflammatory diseases such as celiac disease, irritable bowel syndrome, asthma, and chronic obstructive pulmonary disease.[3] Zonulin levels have also been evaluated within the context of gynecological and obstetric diseases, and increased zonulin levels of the protein have been reported in previous studies of women with a diminished ovarian reserve, gestational diabetes mellitus, and complicated pregnancies in previous studies.[4,5]

Preterm premature rupture of membranes (PPROM) is defined as an early birth following the rupture of fetal membranes before 37 weeks of gestation. It is strongly associated with

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severe adverse obstetric outcomes.[6-8] In most PPROM cases, the etiopathogenesis cannot be precisely explained, but it is thought that an increase in risk factors, immunological factors such as amniocytes, and connective tissue physiology-related factors such as cervical shortening following procedures such as cervical incompetence or conization; often, but not always, bacterial proliferation are involved. The subject is so complex that it is not surprising that we have not been able to solve the balance of pro- and anti-inflammatory activity in pregnancy that allows pregnancy to continue.[9]

Our hypothesis was that elevated plasma zonulin levels contribute to PPROM. We postulated that weight factors and inflammatory processes might be involved in PPROM's etiopathogenesis due to increased intestinal permeability, leading to heightened local inflammation via the translocation of microbial antigens. Consequently, we investigated the association between zonulin levels and PPROM, followed by an examination of the relationship between zonulin levels and birth weight.

METHODS

This prospective case-control study was conducted in the obstetrics clinic of a tertiary care center between January and March 2024. Ethical approval was obtained from the Ankara Etlik City Hospital Scientific Researches Evaluation and Ethics Committee (Date: 10.01.2024, Decision No: AEŞK-BADEK-2024-023). The study adhered to the ethical principles outlined in the Declaration of Helsinki (Edinburgh 2000), and informed consent was obtained from all participants.

Patients diagnosed with PPROM between 32 and 37 weeks were included in the study group, while pregnant women without PPROM in the same gestational week were included in the control group. A total of 44 pregnant women were evaluated, with 22 participants in each group.

Exclusion criteria encompassed obstetric complications (other than PPROM), chronic systemic diseases, chronic bowel disease, gastrointestinal surgery, gastroenteric infections during pregnancy, and smoking.

We recorded and analyzed demographic, obstetric, and clinical features, such as age, gravida, parity, body-mass index (BMI), gestational weeks at delivery, and laboratory tests (white blood cell (WBC) count and C-reactive protein (CRP) at the time of PPROM and at similar weeks of pregnancy for the control group, as well as maternal plasma and umbilical cord blood zonulin levels.

Preparation of Blood Samples

Maternal blood samples were collected upon hospital admission for PPROM in the study group and at the corresponding gestational age in the control group. Umbilical cord blood samples were obtained from all newborns immediately after delivery. These samples were centrifuged at 3000 rpm for 10 min within 24 h and stored at -80°C until analysis. Zonulin levels were determined using a human zonulin ELISA kit (BT LAB-Bioassay Technology Laboratories, Shanghai, China) specifically designed for serum zonulin measurement. Intra- and inter-assay coefficients of variation were below 10%. Serum zonulin concentrations were quantified in ng/ml.

Statistical Analysis

Sample size calculations were performed using G*Power Version 3.1.9.4 (Franz Faul, Universitat Kiel, Germany). Based on a standard effect size of 1.23, a 5% significance level, and 95% power, a power analysis of newborn birth weight data indicated that a minimum of 17 patients per group was required (10). Statistical analyses were performed using IBM SPSS 26.0 software (IBM Corp., Chicago, IL, USA). Variables are expressed as the mean±SD, median (min-max), or number and percentage (n, %). The normality of continuous variables was evaluated by using the Shapiro-Wilk test. The parametric data were compared using a t-test. The nonparametric data were compared using the Mann-Whitney U test. The chi-square test or Fisher's exact test was used to compare categorical variables. Since the data did not follow a normal distribution, the Spearman correlation test was used for all correlation analyses. A p-value of less than 0.05 was considered to indicate statistical significance.

RESULTS

A total of 44 pregnant women, 22 with PPROM (study group) and 22 with non-risk term pregnancy (control group), were included in the study. The demographic and clinical characteristics of the women are shown in Table 1.

Table 1. Demographic and clinical characteristics of the PPROM and control groups

Characteristics	PPROM group (n=22)	Control group (n=22)	p value
Age, year	27.3±6.4	27.9±4.4	0.723
BMI, kg/m^2	28.8±4.2	30.4±7.3	0.366
Gestational age at delivery, weeks	34 w	39 w	<0.001
Gravidity	2 (1-7)	2 (1-10)	0.209
Parity	1 (0-5)	1 (0-4)	0.036
Nulliparous	10 (45.5%)	3 (13.6%)	0.021
Previous abortion	8 (36.4%)	4 (18.2%)	0.176
Previous cesarean delivery	2 (9.1%)	8 (36.4%)	0.031
Previous PPROM	4 (18.2%)	1 (4.5%)	0.345
WBC count at admission, cells/mm^3	11210.0±4287.4	9450.0±2964.1	0.019
Hb level at admission, g/dl	11.5 (9.3-13.8)	11.1 (8.6-13.9)	0.250
PLT level at admission, x10^3/mm^3	295.0 (145.0-424.0)	262.0 (116.0-391.0)	0.330
Number of cesarean deliveries	9 (40.9%)	12 (54.5%)	0.365
Birthweight of newborns, g	2244.7±671.3	3417.7±386.9	<0.001

PPROM: Premature preterm rupture of membrane, BMI: Body-mass index, WBC: White blood cell, Hb: Hemoglobin, PLT: Platelet, The data are presented as the n (%), median (min-max) or mean±standard deviation

No significant difference was found between the PPROM group and the control group regarding age, BMI, gravidity, previous abortion, or history of PPROM. In the PPROM group, 14 patients had CRP levels above 5 mg/dl. The median CRP value was 13.37 mg/dl (range: 6.58-35). Additionally, 18 patients had WBC values above 10,000/µl. The median WBC value was 11,820/µl (range: 10,880-23,130). The median gestational age at delivery was 34 weeks in the PPROM group and 39 weeks in the control group (p<0.001). The percentage of nulliparous women was greater in the PPROM group than

in the control group (45.5% vs. 13.6%, p<0.001). The labor and delivery outcomes of the two groups are shown in Table 1. The cesarean delivery rates of the groups were similar (40.9% vs. 54.5%, p=0.365).

The median maternal plasma zonulin concentrations [99.1 (67.5-307.5) ng/ml vs. 95.6 (65.7-320.0) ng/ml] and cord blood zonulin concentrations [81.9 (30.9-271.3) ng/ml vs. 76.9 (44.1-314.8) ng/ml] were greater in maternal and cord blood in the PPROM group than in the control group, but the differences were not statistically significant (p=0.925 and p=0.681, respectively; Table 2).

Table 2. Zonulin levels in the PPROM and control groups

Zonulin level	PPROM group (n=22)	Control group (n=22)	p value
Maternal blood ng/ml	99.1 (67.5-307.5)	95.6 (65.7-320.0)	0.925
Cord blood ng/ml	81.9 (30.9-271.3)	76.9 (44.1-314.8)	0.681

The data are presented as the median (min-max), PPROM: Premature preterm rupture of membrane

We further divided the participants in the PPROM group into subgroups, with those with a BMI below 30 kg/m² categorized as subgroup 1 and those with a BMI above 30 kg/m² categorized as subgroup 2. Subsequent analysis revealed no difference in maternal serum or fetal cord zonulin levels between the two subgroups (p=0.974).

Based on the demographic data, pregnancy outcomes, and laboratory results, we observed a positive correlation between maternal and fetal cord zonulin levels and newborn birth weight in the PPROM group (p=0.003 and p=0.002, respectively). Furthermore, a comparison of zonulin levels in maternal serum and fetal cord blood between the groups highlighted a strong positive correlation between high zonulin levels in both maternal serum and fetal cord blood (Table 3).

Table 3. Comparison of the correlation between maternal serum and fetal cord blood zonulin levels and their characteristic features

	PPROM cases (n=22)			
	Maternal blood zonulin		Cord blood zonulin	
	r	p	r	p
Age	-0.016	0.944	-0.029	0.898
BMI	0.047	0.835	0.035	0.879
Gestational age	0.185	0.410	0.220	0.325
Birthweight	0.607	0.003*	0.617	0.002*
WBC	-0.115	0.609	0.047	0.837
Hb	-0.084	0.709	-0.143	0.527
PLT	0.085	0.706	0.125	0.580
CRP	-0.031	0.893	-0.067	0.768
Cord blood zonulin level	0.944	<0.001*	-	-

*p values less than 0.05 were considered significant, PPROM: Preterm premature membrane rupture, BMI: Body-mass index, WBC: White blood cell, Hb: Hemoglobin, PLT: Platelet, CRP: C-reaktif protein

However, it was considered that this situation might also stem from differences in the weeks of the newborns. Regression analysis indicated no significant relationship between maternal zonulin levels and BMI (r=0.138, p=0.372) or between newborn birth weight and fetal cord zonulin levels (r=0.211, p=0.170). However, a significant positive correlation was found between maternal zonulin levels and fetal cord zonulin levels (r=0.922, p<0.001), possibly related to intestinal permeability (**Figure**).

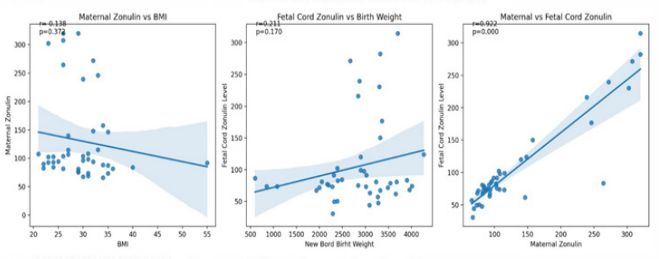

Figure. Distribution of the relationship between zonulin and the factors

A multivariate logistic regression analysis was conducted in a model including statistically significant factors such as parity, previous cesarean, nulliparity, WBC, maternal zonulin levels, and fetal cord zonulin levels. The results are detailed in Table 4. No independent risk factors were identified for PPROM (**Table 4**).

Table 4. Multivariate logistic regression analysis for PPROM

Characteristics	OR (95% CI)
Parity	0.895 (0.429-1.870)
Nulliparous	0.248 (0.029-2.103)
Previous cesarean delivery	2.137 (0.309-14.787)
WBC count at admission	1.000 (1.000-1.000)
Maternal blood zonulin level	1.014 (0.983-1.046)
Cord blood zonulin level	0.989 (0.956-1.024)

PPROM: Preterm premature rupture of membrane, OR: Odds ratio, WBC: White blood cell

DISCUSSION

Previous studies have evaluated the relationship between zonulin and various inflammatory and chronic diseases.[5] However, our study is the first to focus on PPROM patients. We found that zonulin levels did not significantly differ between the PPROM and control groups. However, in the PPROM group, we observed a strong relationship between newborn birth weight and both maternal serum zonulin levels and fetal cord blood zonulin levels. We also found a significant correlation between maternal serum zonulin levels and fetal cord blood zonulin levels in all patients.

Zonulin has been assessed as a new diagnostic marker for intestinal permeability in newborns showing signs of infection and/or inflammation in the gut or at risk of intestinal pathology. Tarko et al.[11] evaluated 81 newborns diagnosed with sepsis, necrotizing enterocolitis, rotavirus infection, or gastroschisis. Zonulin levels did not correlate with CRP or procalcitonin levels in their study. Therefore, the authors suggested that an increase in zonulin might not match the release of inflammatory markers, and that low CRP should not rule out intestinal injury in newborns.[11] In our study, despite the expected development of infection secondary to PPROM, no correlation was found between maternal serum and umbilical cord serum zonulin levels or CRP levels in patients with PPROM. In another study conducted in patients

with preterm rupture of membranes (PROM), zonulin levels were compared between groups with and without PROM. The mean zonulin concentration was greater in the PROM group (155.3±50.2 ng/ml) than in the non-PROM group (128.8±59 ng/ml). However, no statistically significant difference was found between them. Among the inflammatory markers, only C-reactive protein levels were significantly increased in the PROM group.[12] Similarly, in our study, there was no significant difference in zonulin levels between the PPROM group and term pregnant women. However, the level of zonulin, which was high in maternal blood, was also found to be high in cord blood. This finding is significant in terms of intestinal permeability. However, since our study did not routinely measure CRP in term pregnant women, we could not compare its levels between the two groups.

In a study involving 100 healthy newborns and their mothers, zonulin levels in blood samples and calprotectin levels in stool samples were assessed. This study demonstrated that cesarean section delivery and antibiotic use led to an increase in zonulin levels.[13] However, another study reported that long-term cesarean section delivery or antibiotic use did not influence zonulin or other intestinal permeability-related factors.[14] In our study, neither of these two factors resulted in a change in zonulin levels.

For pregnant patients, a weight gain during pregnancy greater than 18 kg or a BMI increase >5.7 during pregnancy is associated with a decrease in zonulin concentrations in the mother's stool and an increase in calprotectin concentrations in the newborn's stool on the seventh day.[13] Changes in maternal BMI during pregnancy can affect intestinal permeability in both the newborn and the mother. The health consequences of increased intestinal permeability in the first days of life are not yet known. Before zonulin and calprotectin tests can be widely used to diagnose increased intestinal permeability, these tests must be validated.[13,15] In our study, analysis of the subjects divided into two groups based on BMI values above and below 30 no significant difference in the comparison of maternal serum and fetal cord blood zonulin levels. This result can be attributed to the lack of a significant difference in BMI values between the study and control groups (28.8±4.2 kg/m^2 vs. 30.4±7.3 kg/m^2) and the presence of only two patients with morbid obesity (BMI=42 kg/m^2 and 55 kg/m^2).

There are numerous studies on the relationship between newborn birth weight and intestinal permeability in various diseases. For instance, in a case-control study involving 368 infants categorized as born below 2500 g and above 2500 g, a notable link was discovered between intestinal permeability and newborn birthweight based on maternal serum zonulin levels.[10] The serum levels of zonulin and zinc in mothers of infants weighing more than 2500 g were found to be significantly greater than those in mothers of low-birth weight infants.[10] In our study, we found a positive correlation between newborn birthweight and maternal serum zonulin levels and fetal cord blood levels in the PPROM group (Table 2). In our study, a positive correlation was found between maternal serum zonulin levels and fetal cord zonulin levels (Table 3). This correlation suggests that changes in zonulin levels may lead to complicated pregnancies with impaired glucose tolerance, insulin resistance, gestational diabetes mellitus, and intrahepatic cholestasis, as indicated in the literature.[16,17]

In complex diseases such as polycystic over syndrome and PROM, which can present with metabolic disorders, gestational diabetes mellitus, or chronic inflammatory bowel diseases, the expected increase in zonulin levels could not be observed in the literature. This is attributed to the unclear relationship between the mechanism of action of zonulin and these diseases.[12,18-20]

Limitations

The major strength of the present study is that it is the first, to our knowledge, to investigate the association between PPROM and zonulin levels. The study is also strengthened by its prospective design. Conversely, the small sample size is a significant limitation. Due to the sample size of obese women, a significant association could not be demonstrated between BMI and zonulin levels.

CONCLUSION

No significant association was found between PPROM and maternal serum/umbilical cord blood zonulin levels in this study. This outcome is attributed to the clinical presentation of PPROM, which is characterized by a localized inflammatory process rather than a systemic process. However, a positive correlation was found between newborn birthweight and maternal serum zonulin levels, as well as fetal umbilical cord levels, in the PPROM group. There was also a positive correlation between maternal serum and fetal umbilical cord zonulin levels in all patients.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of the Ankara Etlik City Hospital Scientific Researches Evaluation and Ethics Committee (Date: 10.01.2024, Decision No: AEŞK-BADEK-2024-023).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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