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The Evaluation of Intestinal Permeability in Preeclamptic Pregnancy

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ABSTRACT

Background: Zonulin is a physiological protein that regulates the tight connections and permeability of the intestine, serving as a biomarker for impaired intestinal permeability. **Objective:** The aim of this study was to examine zonulin levels in preeclampsia, to investigate its associations with the cellular immune response marker soluble interleukin-2 receptor (sIL-2R) and exogenous antigen load marker lipopolysaccharide binding protein (LBP) and to evaluate the implications of these findings in the etiopathogenesis of preeclampsia. **Methods:** We designed a cross-sectional case-control study and enrolled 22 pregnant women with preeclampsia and 22 healthy pregnant controls. Plasma zonulin levels were determined by ELISA. Serum sIL-2R and LBP levels were assessed by chemiluminescent immunometric methods. **Results:** Women with preeclampsia had lower levels of plasma zonulin and serum LBP than normotensive healthy controls (p<0,05). The difference in serum sIL-2R levels was not significant (p: 0,751). There was a negative correlation between plasma zonulin and serum urea (r: -0.319, p: 0.035) and a positive correlation between serum sIL-2R and ALT (r: 0,335, p: 0,026) and AST (r: 0,319, p: 0,035). **Conclusion:** We found that zonulin and LBP, but not sIL-2R, levels were significantly lower in pregnant women with preeclampsia as compared with healthy pregnant controls. Reduced intestinal permeability in preeclampsia might be associated with impaired immune system functions or a lower fat mass and malnutrition. Further studies are needed to elucidate the exact pathogenetic role of intestinal permeability in preeclampsia.

Keywords: preeclampsia, intestinal permeability, zonulin, lipopolysaccharide binding protein, IL-2 receptor.

1. BACKGROUND

Preeclampsia is a pregnancy-specific disorder characterized by a systemic disease process comprising impaired placental perfusion and multiple system dysfunctions. According to an update by the International Society for Hypertension Pregnancy (ISSHP) Association in 2014, preeclampsia is defined as de-novo hypertension that occurs after the 20th week of pregnancy along with proteinuria and/or other maternal organ dysfunctions such as renal failure, liver dysfunction, neurological and/or hematological complications (1). It is one of the leading causes of maternal, fetal, and neonatal death in underdeveloped and developing countries (2).

Over the past 20 years, the incidence of preeclampsia has increased steadily largely due to an increase in risk factors like chronic hypertension, diabetes, obesity, advanced maternal age and the use of assisted reproductive technology (3, 4). Additionally, recent research indicates that immunological mechanisms may also play a significant role in the development of preeclampsia (5). Among these, gut barrier dysfunction has been suggested as a potential risk factor. However, few studies have explored its role in the etiology of preeclampsia. One study suggested that a mother's diet pattern could contribute to the development of preeclampsia because of its effects on gut microbiota (6). Another one indicated that abdominal compartment syndrome could lead to increased intestinal permeability, and trigger a systemic inflam-

	Pregnant women with preeclampsia (n:22)	Healthy pregnant women (n:22)	Test value; p
Parameter	Mean ± Sd or Median (%5-95)	Mean ± Sd or Median (%5-95)	
Age (year)	29,818 ± 6,26	29,36 ± 5,17	0,794
Height (cm)	159,14 ± 6,77	157,45 ± 4,5	0,338
Weight (kg)	79,59 ± 16,74	80,59 ± 12,25	0,822
BMI (kg/m^2)	31,27 ± 5,5	32,46 ± 4,44	0,436
Nulliparity (n, %)	8 (%36,4)	4 (%18,2)	0,310
Multiparity (n, %)	14 (%63,6)	18 (%81,8)	
Gestation age (week)	33,64 (22.28-37,94)	35,46 (24,36-38,71)	0,071
Systolic Blood Pressure (mmHg)	145,0 (140,0-197,0)	110,0 (100,0-134,25)	0,000*
Diastolic BP (mmHg)	90,0 (90-125,5)	70,0 (60,80,0)	0,000*

Table 1- Demographic and clinical data of the study and control groups. * Significance: p<0.05

	Pregnant women with preeclampsia (n:22)	Healthy pregnant women (n:22)	Test value; p
Parameter	Mean ± Sd or Median (%25-75 percentile)	Mean ± Sd or Median (%25-75 percentile)	
Fasting glucose (mg/dL)	84,55 ± 13,714	80,23 ± 10,726	0,251
Alanine aminotranspherase (ALT) (U/L)	13,5 (7,0 -37,5)	11,0 (6,0- 49,70)	0,65
Aspartate aminotranspherase (AST) (U/L)	18,0 (12,0-40,10)	17,0 (9,0-51,25)	0,488
Lactate dehydrogenase (LDH) (U/L)	203,5 (155,30-356,75)	179,5 (128,75-354,45)	0,269
Urea (mg/dL)	19,5 (11,15-53,55)	13 (9,0-28,80)	0,02*
Leukocyte (10^3/µL)	11,05 (7,515-21,745)	9,850 (6,705-18,725)	0,226
Hematocrit (%)	33,555 ± 3,5378	33,609 ± 3,3235	0,958
Hemoglobin (g/dL)	11,15 (9,515-13,25)	11,65 (8,5-12,67)	0,672
Platelet (10^3/µL)	233,68 ± 57,68	211,80 ± 72,451	0,274
Mean Platelet volume (MPV) (µm^3)	9,982 ± 2,196	9,673 ± 1,446	0,584

Table 2- Laboratory values of the study and control groups. * Significance: p<0.05

	Pregnant women with preeclampsia	Healthy pregnant women	Test value; p
Parameter	Median (%25-75 percentile)	Median (%25-75 percentile)	
Zonulin (ng/mL)	14,833 (13,86-16,15)	16,837 (14,2-21,2)	0,044*
LBP (µg/mL)	11,35 (8,675-14,915)	14,9 (11,575-16,362)	0,017*
sIL-2R (U/mL)	451,5 (259,25-657,62)	396,75 (283,37-602,87)	0,751

Table 3- Comparison of test parameters between patients with preeclampsia and healthy pregnant women* Significance: p<0.05

matory response, possibly resulting in preeclampsia (7).

We conducted a study aimed to first assess the intestinal epithelial membrane permeability in preeclampsia and subsequently examined the association between gut permeability and systemic inflammation using several biochemical and inflammatory markers.

2. OBJECTIVE

The aim of this study is to examine zonulin levels, which is an intestinal epithelial permeability marker, in preeclampsia, to investigate its associations with the cellular immune response marker soluble interleukin-2 receptor (sIL-2R) and exogenous antigen load marker lipopolysaccharide binding protein (LBP) and to evaluate the implications of these findings in the etiopathogenesis of preeclampsia.

3. PATIENTS AND METHODS

Participants

We conducted a cross-sectional and case-controlled study in pregnant women presenting to the gynecology and obstetrics department with a diagnosis of preeclampsia and in healthy pregnant women between 22 and 40 weeks of gestation.

Pregnant women with conditions such as diabetes, chronic inflammatory bowel disease, rheumatoid disease, or acute inflammatory diseases such as tonsillitis, urinary tract infection, chorioamnionitis, etc. were excluded from the study. Patients with a history of cardiovascular disease, hypertension, and those who received steroid treatment in the last 6 months were also excluded. The control group consisted of healthy pregnant women who received regular follow-up care at the same obstetrics and gynecology clinic. Both study and control groups were matched for age and BMI with no significant differences ($p > 0.05$).

Procedure and ethical considerations

The study protocol was approved by the ethics committee of Istanbul Kartal Dr. Lütfi Kirdar Training and Research Hospital and we obtained informed consent from all participating subjects. Each participant was informed about the purpose of the study. The Helsinki Declaration of 1983's ethical standards were followed, and all participants were informed that they had the option to decline or discontinue their participation.

Measures

Blood pressure was recorded after a minimum rest period of 10 minutes while the person was seated. Pre-

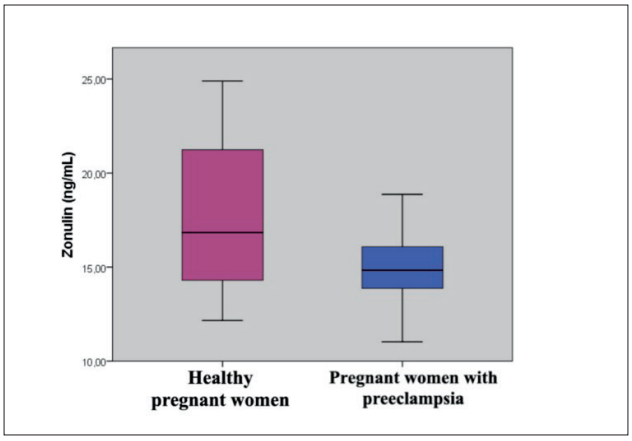

Figure 1. Zonulin level distribution in pregnant women with preeclampsia and healthy women.

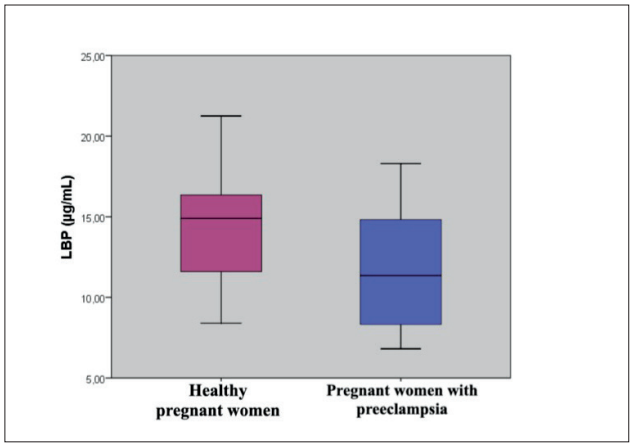

Figure 1. LBP level distribution in pregnant women with preeclampsia and healthy women.

eclampsia diagnosis was made using the ISSHP criteria and applied to pregnant women after 20 weeks of gestation who had de novo hypertension (>140 / 90 mmHg) and either proteinuria (≥ 300 mg/24 h and/or protein/creatinine ratio in spot urine ≥ 0.3 and/or urine protein dipstick level ≥+1, thrombocytopenia (platelet count < 100.000/µL), renal failure (creatinine level > 1.1 mg/dL), impaired liver function (transaminase values twice as high as the reference values), or any cerebral symptoms such as headache, visual disturbances, convulsion, or pulmonary edema.

We used the semi-automatic ELISA method to measure zonulin using the SunRed Human Zonulin ELISA kit (catalog no: 201-12-5813) from Shanghai Sunred Biological Technology Co. Ltd in China, following the kit procedure.

The measurement of lipopolysaccharide binding protein (LBP) was performed using the chemiluminescent immunometric assay on the Immulite® 2000 XPI Immunoassay autoanalyzer from Siemens Healthcare Diagnostics Products Ltd., UK, with a kit provided by the same company (catalog number L2KLB2, UK).

We measured soluble interleukin-2 receptor (sIL2R) using the chemiluminescent immunometric method on Immulite® 2000 XPI Immunoassay autoanalyzer (Siemens Healthcare Diagnostics Products Ltd., UK) with a kit from Siemens Healthcare Diagnostics Products Ltd. (catalog no: L2KIP2, UK)

Statistical analysis

For statistical analysis, we used SPSS Inc's Statistical Package for the Social Sciences® v23.0 software (located in Chicago, Illinois, USA). Categorical variables were analyzed through the Chi-square test, and the Kolmogorov – Smirnov test was used to determine if the distribution of continuous variables was normal. If the parameters showed a normal distribution, we utilized the Student's t-test, but if they didn't, the Mann – Whitney U-test was used instead. The Pearson correlation test and linear regression analysis were used to determine any linear connections between the markers and the continuous variables.

4. RESULTS

Demographic and Clinical Characteristics

The study consisted of 22 pregnant women with preeclampsia aged 17 to 42 and 22 normotensive healthy pregnant women. The demographic and clinical data of both groups is presented in Table 1. The study and control groups were well-matched for age and BMI ($p > 0.05$).

No significant difference was found in glucose, ALT, AST, LDH, leukocyte, hematocrit, hemoglobin, platelet and MPV values between the groups. However, there was a significant difference in urea values ($p < 0.05$) (Table 2). Plasma zonulin and serum LBP levels were significantly lower in pregnant women with preeclampsia compared to healthy pregnant women in the control group (Table 3) (Figure 1 and 2). We identified a moderate negative correlation between plasma zonulin concentration and serum urea (r: -0.319, p: 0.035) and a moderate positive correlation between serum sIL-2R and ALT and AST (ALT r: 0.333, p: 0.026; AST r: 0.319, p: 0.035). The results of the regression analysis showed that only age had an independent effect on LBP levels.

5. DISCUSSION

We found that pregnant women with preeclampsia had significantly lower levels of zonulin and LBP, but not sIL-2R, compared to healthy pregnant controls. This contradicts some previous study and we will discuss the possible causes.

To date, zonulin remains the only known physiological protein that regulates the tight connections and permeability of the intestine, serving as a biomarker for impaired intestinal permeability (8). The current study is the first one to show that serum zonulin and LBP levels were significantly lower in pregnant women with preeclampsia than in healthy pregnant women. Contrary to our findings, several studies have shown an increase in zonulin levels associated with either immunological abnormalities, intra-abdominal pressure increase or genetic predisposition and resulting in gut leakage through the epithelial barrier, triggering the release of pro-inflammatory cytokines (9–12).

The presence of low levels of zonulin and LBP in pregnant women with preeclampsia may suggest a defect

in the immune system. Several studies suggested that opening the paracellular pathway by zonulin serves as a defense mechanism, preventing bacterial colonization in the small intestine by exposing microorganisms to the natural immune system (13, 14). Another study, by Hunt et al., showed that low zonulin levels may be a result of intestinal epithelial cell death, and that they were a strong predictor of mortality in immunocompromised patients (15).

The low levels of zonulin in our study may also be partially due to not taking into account the presence of edema, which is more severe in pregnant women with preeclampsia (16). A 2013 study by Zak-Golab et al found that plasma zonulin levels were higher in obese patients compared to those with normal weight (17). Aasbrenn et al showed that zonulin concentration decreased after weight loss (18). Although both groups in our study were similar in terms of BMI, the actual body fat percentage of pregnant women with preeclampsia could be lower than healthy pregnant women due to edema. The presence of symptoms like reduced appetite, poor nutrition, and vomiting in pregnant women with preeclampsia may also support this possibility (19).

LBP is a plasma protein that binds to lipopolysaccharides (LPS) which are a component of the outer membrane of Gram-negative bacteria. It is used as a marker of systemic inflammation and is involved in the activation of the immune response to bacterial infections (20-23). Currently, limited research exists on the relationship between LBP and preeclampsia, with only one small study of 13 pregnant women with preeclampsia showing higher LBP levels (24). Low levels of LBP in the current study may be related to the decrease in zonulin levels.

Interleukin-2 (IL-2) is a cytokine that plays a crucial role in regulating the immune system, and sIL-2R is a form of IL-2 receptor that exists in the soluble form. It is found in the bloodstream and acts as a negative regulator of the IL-2 signaling pathway. Elevated levels of sIL-2R are associated with various inflammatory and autoimmune diseases and used as a marker of disease activity in these conditions. sIL-2R values did neither show any significant increase in the current study nor did they show a correlation with zonulin or LBP levels.

Limitation of the study

This study has several limitations. The sample size is relatively small, and our results therefore require confirmation through larger studies. While our results already give an idea over the potential relationship between gut permeability and preeclampsia, the relationship between gut permeability and systemic inflammation in pregnant women with preeclampsia can be further assessed with a wider set of systemic inflammatory markers.

6. CONCLUSION

To conclude we found that zonulin and LBP, but not sIL-2R, levels were significantly lower in pregnant women with preeclampsia as compared with healthy pregnant controls. Reduced intestinal permeability in preeclampsia might be associated with impaired immune system functions and/or a lower fat mass and malnutrition. Further studies are needed to elucidate the exact pathogenetic role of intestinal permeability in preeclampsia.

- **Patients Consent Form:** All participants were informed about subject of the study.
- **Authors contribution: All** G.M. conceived of the presented idea, developed the theory, designed and performed the experiments, and took the lead in writing the manuscript. T.Y. contributed to patient selection A.B.G. contributed to sample preparation. O.C.M. helped supervise the project. A.O.K. contributed to the final version of the manuscript.
- **Conflict of interest:** There are no conflicts of interest
- **Financial support and sponsorship: Nil.**

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