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Do the levels of hepcidin and tenascin-C change in patients with preterm premature rupture of membranes? Can these markers help in diagnosis?

Hepcidin and tenascin-C levels in preterm premature rupture of membranes

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Abstract

Aim: In this study, it was aimed to investigate the role of tenascin-C and hepcidin levels in the diagnosis of preterm premature rupture of membranes (PPROM).

Material and Methods: This study is a prospective cohort study conducted at Van Yuzuncu Yil University (YYU), Dursun Odabası Medical Center Gynecology and Obstetrics outpatient clinic between 31.03.2021 and 15.03.2022. One hundred patients aged 18 to 45 years diagnosed with PPROM at 24 to 37 weeks of gestation were included in the study as the "case group", and 100 healthy pregnant women were included as a "control group". Demographic data and laboratory parameters of the patients, such as C-reactive protein (CRP), leukocyte, neutrophil, hepcidin, and tenascin-C levels, were recorded.

Results: Compared to the control group, CRP, leukocytes, and neutrophil levels were statistically significantly higher in the PPROM group ($p<0.05$). Hepcidin value was statistically significantly lower in the PPROM group compared to the control group ($p = 0.003$); tenascin-C levels between the two groups were not statistically different ($p = 0.161$).

Discussion: Hepcidin levels can be used in the diagnosis of PPROM. Multicenter randomized controlled studies with more cases and parameters are needed.

Keywords

Prematurity, Rupture of Membranes, Inflammation, Hepcidin, Tenascin-C

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Introduction

PPROM is defined as rupture of membranes occurring before 37 weeks of gestation [1]. The incidence of premature rupture of membranes occurs in approximately 5% to 10% of all births [2]. Although risk factors for PPROM include a history of PPROM in a previous pregnancy, short cervical length, bleeding during pregnancy, cigarette smoking, malnutrition, low socioeconomic status, the most common risk factors are intrauterine infections and inflammation. A woman with premature rupture of membranes has an increased risk of chorioamnionitis, placental abruption, retained placenta, sepsis, postpartum infection, endometritis, and post-partum hemorrhage [3-5].

Hepcidin is a peptide hormone that can inhibit iron absorption and transport and also plays a role in the anti-microbial function and host defense [6]. It regulates iron transfer via the placental syncytiotrophoblast during pregnancy [7]. It is thought that the increase in maternal and fetal iron demand in the third trimester leads to a decrease in hepcidin levels [6,8]. However, increased hepcidin levels were associated with increased inflammation in pregnant women [9,10].

Tenascin-C is an extracellular matrix glycoprotein [11]. It takes part in shaping stem cells in both inflammatory and fibrotic processes in the tissue damage process. High tenascin-C levels have been reported during the healing of scar tissue, cancer development, and cardiovascular diseases [12]. Although there is no study that establishes a relationship between tenascin-C and inflammation, it has been suggested that tenascin-X levels from the same family may be associated with inflammation [13-15].

Preterm premature rupture of membranes is a clinical picture with high morbidity and mortality, and many possible undesirable outcomes can be prevented by early diagnosis. In this respect, biomarkers that can make a difference in the diagnosis of PPROM other than classical anti-inflammatory markers are needed in clinical practice. In this study, we aimed to find the place of hepcidin and tenascin-C values in the diagnosis of PPROM.

Material and Methods

Ethical approval for the study was obtained from the Van YYU Non-Interventional Clinical Research Ethics Committee on 2021-03-31 with decision No. 02. Informed consent was obtained from the patients, stating that they voluntarily provided the information used.

The research is a prospective cohort study conducted at Van YYU, Dursun Odabasi Medical Center Gynecology and Obstetrics outpatient clinic between 31.03.2021 and 15.03.2022. On the specified dates, 100 pregnant patients diagnosed with PPROM between 24-37 weeks of gestation were included in the study as a "case group", and 100 healthy pregnant women were included in the study as a "control group".

Patients younger than 18 and older than 45 were not included in the study. Also, patients with active infection at the time of diagnosis, pregnant women with chronic diseases and patients with systemic complications such as preeclampsia were excluded from the study.

Data were collected using a data collection form, which was created by examining previous studies on the subject and considering our clinical experience. Demographic data of the pregnant women (age, gestational week, body mass index (BMI), gravida, parity, mode of delivery) and laboratory parameters (C-reactive protein (CRP), leukocyte and neutrophil counts, hepcidin and tenascin-C levels) were recorded for each patient. The recorded weeks of gestation were calculated using the date of the last menstrual period and were confirmed by early first-trimester ultrasonography. The diagnosis of preterm premature rupture of membranes was made by monitoring active amniotic fluid inflow within the speculum or by positive placental alpha microglobulin-1 test.

Venous blood (5 ml) from each patient participating in the study was taken into a biochemistry tube and centrifuged at 4000 rpm for 10 minutes and then stored at -20 degrees in two Eppendorf tubes. After this blood was brought at room temperature under appropriate conditions, hepcidin and tenascin-C kits were used for these samples, and the levels of these peptides were recorded. The analyses of the study were carried out in the SPSS 21.0 package program. Categorical variables were shown as numbers and percentages, and continuous numerical variables as mean, standard deviation, median, minimum and maximum values. The value of numerical parameters in predicting PPROM was evaluated using ROC analysis. The conformity of continuous numerical variables to normal distribution was evaluated using the Kolmogorov-Smirnov test. It was observed that the normal distribution assumption was not met in the distribution of continuous numerical variables between groups, and therefore nonparametric tests were used. The Mann-Whitney U Test was used to compare numerical parameters between two independent groups. The most ideal cutoff point and sensitivity specificity values for significant parameters were determined using the Youden Index, and the ROC curve graph of the parameters was presented.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

A total of 200 pregnant women, 100 healthy and 100 with PPROM, who met the inclusion criteria of the study, formed the study group. All of the pregnant women were between the ages of 18-45, and the mean age was 28.13 ± 5.94 years, and the mean week of gestation was 31.41 ± 4.12 weeks. There was no statistically significant difference between the groups in terms of age, gestational week, BMI, gravida and parity values ($p>0.05$) (Table 1). Again, the delivery types did not differ between the two groups, and 27% of the control group and 23% of the case group required cesarean section ($p = 0.514$).

Compared to the control group, CRP, leukocyte, and neutrophil levels were statistically significantly higher, and hepcidin levels were significantly lower in the PPROM group ($p<0.05$). Tenascin-C levels were not significantly different between the groups ($p = 0.161$). The mean hepcidin value was 41.03 ± 1.66 ng/mL in the control group and 37.57 ± 19.31 ng/mL in the PPROM group. The hepcidin value was statistically significantly lower in the PPROM group compared to the control group ($p = 0.003$) (Table 2).

The graphical representation of the distribution of hepcidin value among the groups is given below (Figure 1).

Table 1. Comparison of age, gestational week, BMI, gravida and parity between PPROM and control groups.

Variables	Control (n = 100)		PPROM (n = 100)		P
	Mean±SS	Median (min-max)	Mean±SS	Median (min-max)	
Age (year)	27.66±5.87	27 (18-40)	28.6±5.99	28 (18-45)	0.31
Gestational week	31.47±3.73	31.4 (24-37)	31.35±4.49	32.45 (24-37)	0.954
BMI (kg/m²)	21.64±0.99	21.5 (20-24)	21.61±1.7	21 (18-26)	0.418
Gravida	2.96±1.73	3 (1-7)	3.03±2.18	2 (1-10)	0.667
Parity	1.71±1.6	1 (0-6)	1.68±1.73	1 (0-7)	0.708

BMI, body mass index; PPROM, preterm premature rupture of membranes.
Mann-Whitney U Test was used for comparisons.

Table 2. Comparison of laboratory results between PPROM and control group.

Variables	Control (n = 100)		PPROM (n = 100)		P
	Mean±SS	Median (min-max)	Mean±SS	Median (min-max)	
CRP (mg/L)	6.23±5.41	4.1 (2-39.1)	12.22±16.55	7.33 (2-129)	0.010
Leukocyte (10^9/L)	10.41±2.53	10.17 (5.57-20.5)	13.18±4.6	12.15 (7.18-31.5)	<0.001
Neutrophil (10^9/L)	7.88±2.31	7.64 (3.63-17.5)	10.78±4.62	9.88 (4.66-28.2)	<0.001
Hepcidin (ng/mL)	41.03±17.66	40.16 (12.98-157.66)	37.57±19.31	34.47 (11.44-123.2)	0.003
Tenascin-C (pg/mL)	4.29±1.89	4.04 (1.25-13.38)	3.85±1.64	3.91 (1.05-10.34)	0.161

CRP, C-reactive protein; PPROM, Preterm premature rupture of membranes.
Mann-Whitney U Test was used for comparisons.

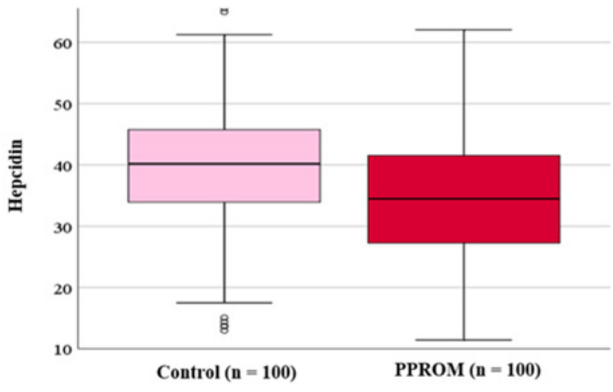

Figure 1. Distribution of the hepcidin levels between the groups.

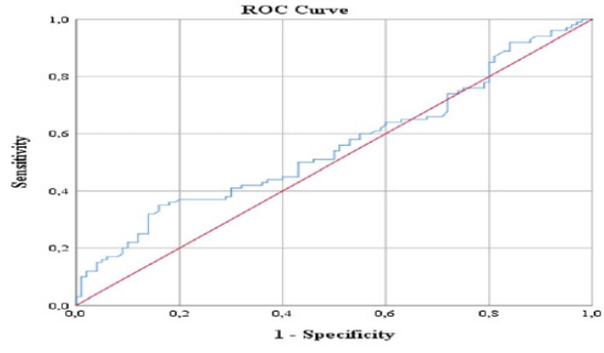

Figure 2. ROC curve graph of tenascin-C levels in terms of predicting PPROM.

The ROC curve graph of the tenascin-C level in terms of predicting PPROM is shown below. Tenascin-C level was not found to be significant in the prediction of preterm premature rupture of the membranes (Figure 2).

Discussion

As known, CRP is an important indicator of inflammation. Intrauterine inflammation is considered to be the most important cause of PPROM among the multiple mechanisms involved in PPROM. Stating that inflammation is one of the most important causes of PPROM, Ekin et al. reported that the serum level of parameters originating from inflammation increased significantly in PPROM cases [16]. Banaem et al. in their prospective cohort study in which CRP levels were recorded in 778 pregnant women, reported that CRP levels of the cases with PPROM were significantly higher compared to the other pregnant women [17]. The result of our study is also compatible with previous studies showing the relationship between CRP and PPROM. Like many previous studies, our study also confirms that CRP can recognize PPROM cases.

In a study conducted in 2021, Iflazoğlu et al. compared the results of PPROM cases and pregnant women in the control group, and reported that the leukocyte count of PPROM cases was significantly higher compared to healthy pregnant women [18]. Lyubomirskaya et al. reported that leukocyte and neutrophil count could predict PPROM in their study in which they compared the results of PPROM cases and healthy pregnant women [19]. Ramya et al. reported in another study that leukocyte and CRP levels were significant in both differentiating cases with PPROM from healthy pregnancies and in predicting intra-amniotic infection [20]. Balciuniene et al. in a study they conducted in 2021 showed that neutrophil/leukocyte levels were significantly increased among PPROM cases [21]. The significantly higher leukocyte and neutrophil counts of PPROM cases detected in our study compared to healthy pregnant women are consistent with the results of many previous studies. Just like CRP, we think that the increase in leukocytes and neutrophils is mainly due to the presence of an inflammatory process in PPROM cases.

There are very few studies associated with PPROM for hepcidin, one of the main markers of our study. In a study by Simavli et al., it was reported that there was a significant relationship between hepcidin level and increased acute phase reactants in pregnant women; this has been interpreted as that hepcidin may be a parameter indicating inflammation in pregnant women [9]. Onaran et al. compared prohepcidin levels between term and preterm birth cases and showed that maternal serum prohepcidin levels decreased among cases with preterm labor [22]. In the study conducted by Fırat et al., hepcidin levels were assessed to be high in pregnant women with preterm labor and it was reported that it might have the potential to be used as biological markers [10]. Bencalova et al. emphasized that increased hepcidin level may be associated with adverse pregnancy outcomes, including PPROM [23]. Our study showed that the hepcidin level is significant in distinguishing cases with PPROM from healthy pregnant women. Based on this result, we think that it would be beneficial to conduct studies examining the relationship between early-stage hepcidin levels of pregnant women and the development of PPROM in the advancing

gestation, and possible mechanisms in future studies.

Another parameter that compared serum levels between groups in our study was tenascin-C level, and tenascin-C levels were found to be similar between PPROM and healthy pregnancy groups. There are some studies showing that tenascin-X, another peptide belonging to the tenascin family, increases the risk and frequency of PPROM [13-15]. Looking at the literature, there is no other study that directly evaluates the relationship between tenascin-C and PPROM.

The blood values of the cases were examined after the development of PPROM in our study; therefore, temporal characteristics such as whether the results were secondary to PPROM or before the development of PPROM could not be evaluated, and no interpretation could be made in this respect due to the study design. A study where hepcidin, for which there are many studies showing that its level decreases towards the last trimester, especially during the normal pregnancy course, will be evaluated in the first trimester and where these patients will be followed for the next trimesters in terms of PPROM, may contribute significantly to the prediction of PPROM.

Conclusion

While hepcidin level was statistically significantly higher in cases with PPROM; tenascin-C level did not make a significant difference compared to normal patients in this respect. Randomized controlled studies are needed to evaluate the effect of hepcidin in predicting PPROM, the role of tenascin-C level in PPROM, and even the relationship of these markers, measured at early gestational weeks, with PPROM.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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