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# Predictive Role of Osteopontin and Inflammation Markers in the Diagnosis and Monitoring of Premature Membrane Rupture

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## ABSTRACT

**Objective:** Preterm premature rupture of membranes (PPROM) is rupture of membranes before 37 weeks of gestation. It is the most common cause associated with preterm labor, accounting for about one-third of preterm births. Osteopontin is a phosphorylated and glycosylated protein consisted of 264-301 amino acids. It is an extracellular matrix member. It has been observed in various biological fluids, epithelial cells, gastrointestinal system secretions, kidneys, thyroid, breast, uterus, placenta and testis. The aim of this study was to investigate whether there is a significant relationship between maternal serum osteopontin levels and PPROM. Also, this study aimed to evaluate the role of osteopontin in the prediction and appropriate management of PPROM.

**Materials and Methods:** This prospective cross-sectional study was conducted in the Gynecology and Obstetrics Clinic of Dr. Sami Ulus Obstetrics, Gynecology and Pediatrics Training and Research Hospital. The study group consisted of pregnant women who were hospitalized due to PPROM and the control group consisted of healthy pregnant women who were followed up in outpatient clinic. For biochemical analysis, fifteen milliliters venous blood samples were taken from all participants. Plasma Osteopontin and CRP values, and sedimentation rate were measured.

**Results:** A total of 64 pregnant women, 32 patients in each group, were included. Serum leukocyte, sedimentation, C reactive protein and osteopontin levels of pregnant women in the control group were significantly lower than those of participants in the PPROM group.

**Conclusions:** Serum Osteopontin levels increase in patients diagnosed with PPROM. Measuring serum Osteopontin levels may be used as an important marker in the prediction and management of PPROM.

**KEYWORDS**
Osteopontin; inflammation; premature membrane rupture; pregnancy.

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## Introduction

Premature rupture of membranes (PROM) is the rupture of membranes before uterine contractions begin. Preterm premature rupture of membranes (PPROM) is rupture of membranes before 37 weeks of gestation. It is the most common cause associated with preterm labor, accounting for about one-third of preterm births[1]. PPROM occurs in 3% of pregnancies, of which approximately 0.5% are <27 weeks, 1% between 27 and 34 weeks and 1% between 34 and 37 weeks. Various pathological events (sub-clinical or overt infection, inflammation, mechanical stress, hemorrhage, etc.) can activate a cascade that may result in PROM[2]. Many maternal physiologic, genetic and environmental factors probably predispose to the development of PPROM in many cases. The diagnosis of PPROM is made clinically and is usually based on the visualization of amniotic fluid in the vagina of a woman with a history of fluid leakage. In the cases of suspicious, laboratory tests can be used to confirm the clinical diagnosis[3].

Approximately 1/3 of women with PPROM develop potentially serious infections such as intra-amniotic infection, endometritis or septicemia. The fetus and newborn are at greater risk of PPROM-related morbidity and mortality than the mother. It was reported that fetal exposure to intrauterine inflammation has been associated with an increased risk of neuro-developmental impairment[4].

Osteopontin (OPN) is a phosphorylated and glycosylated protein consisted of 264-301 amino acids. It is an extracellular matrix member. Post-transcriptional modification is required for its activity[5]. It has been observed in various biological fluids, epithelial cells, gastrointestinal system secretions, kidneys, thyroid, breast, uterus, placenta and testis[6]. It is thought that OPN, a secretory product of uterine glandular epithelium, may affect fetal placental development and growth; and may work together with progesterone in various pathways within this system[7].

The aim of this study was to investigate whether there is a significant relationship between maternal serum osteopontin levels and PPROM. Also, this study aimed to evaluate the role of osteopontin in the prediction and appropriate management of PPROM.

## Material and Methods

The study was conducted after the approval from Ankara Keçiören Research Hospital Clinical Researches Ethics Committee (Date: 28.02.2018, issue number: 2012-KAEK-15/1624). This prospective cross-sectional study was carried out in the Gynecology and Obstetrics Clinic of Dr. Sami Ulus Obstetrics, Gynecology and Pediatrics Training and Research Hospital between the dates of 01.03.2018 and 01.07.2018.

The study group consisted of pregnant women who were hospitalized due to PPROM and the control group consisted of healthy pregnant women who were followed up in outpatient clinic. Informed written consent was obtained from all patients before inclusion in the study. Patients between 24-37 weeks of gestation were included. Those with multiple pregnancies, comorbidities such as preeclampsia, diabetes, and psychiatric problems were excluded from the study.

The diagnosis of PPROM was made clinically by observing the flow of active amniotic fluid, and in suspicious cases, the diagnosis was confirmed with the nitrazine test or amnisure test. Three venous blood samples, two 5 mL samples for osteopontin and CRP levels measurements and a 5 mL sample for sedimentation measurement, were taken from all pregnant women in EDTA tubes. These blood samples were centrifuged at 2000 rpm for 10 minutes and separated into Epondorf tubes as appropriate, plasma samples were stored in a deep freezer at -80 degrees until

the day of study until they were examined. Plasma Osteopontin and CRP values were measured by the Enzyme-Linked Immunosorbent Assay (ELISA) method using a kit (Hummonosteopontin assay kit, Atlas Biotechnology)[8]. Total Osteopontin levels in phosphorylated and non-phosphorylated forms in plasma were measured. Osteopontin and CRP levels in each serum sample were calculated by making regression-correlation analysis with Microsta, a computer-based statistical program, using the OD (optical density) values of calibrators with known concentrations. Sedimentation rate was measured by the Westergren method.

The age, height, weight and obstetric history of the pregnant women included in the study were recorded. Information about birth, type of birth, and birth weight were obtained retrospectively from the hospital registry system. Statistical analyzes were performed using the package program SPSS (IBM SPSS Statistics 20). Mean Standard Deviation, Median, Minimum, Maximum values and percentage values were used in descriptive statistics for continuous data. Data with normal distribution were analyzed with the independent sample t test method, and data with non-normal distribution were analyzed with the Mann Whitney U test. Chi-square test was used to examine the relationships between two variables. In all analyses, the statistical significance level was accepted as 0.05.

## Results

A total of 64 pregnant women, 32 patients in each group, were included in the study. No statistical difference was observed between the groups in terms of age, BMI, fetal weight and obstetric history. The gestational weeks of pregnant women in the control group were smaller than those in the PPROM group (Table 1). Serum leukocyte, sedimentation, C reactive protein and osteopontin levels of pregnant women in the control group were significantly lower than those of participants in the PPROM group (Table 2, Figure 1).

**Table 1. Demographic characteristics of the patients.**

| | PPROM Group (n=32) | Control Group (n=32) | P value |
|---|---|---|---|
| Age (years) mean±SD | 26.84±5.11 | 26.81±5.28 | 0.981 |
| BMI (kg/m$^2$) | 28.68±2.87 | 27.25±2.97 | 0.053 |
| Gravidity [med. (min-max)] | 1 (1-6) | 2 (1-4) | 0.902 |
| Parity [med. (min-max)] | 0 (0-5) | 0 (0-2) | 0.982 |
| Abortion [med. (min-max)] | 0 (0-3) | 0 (0-1) | 0.905 |
| Gestational week (mean±SD) | 33.50±3.57 | 32.29±3.07* | 0.019 |
| Fetal weight (mean±SD) | 2269.34±629.39 | 2068.1±684.15 | 0.283 |

*p<0.05, compared to PPROM Group.

**Table 2. Comparison of biochemical analysis results of groups.**

| | PPROM Group (n=32) | Control Group (n=32) | P value |
|---|---|---|---|
| Leukocyte ($10^3/mm^3$) | 14.98±4.59 | 11.00±2.06* | <0.001 |
| Sedimentation (mm/hour) | 45.28±15.68 | 28.25±6.92* | <0.001 |
| C reactive protein (mg/dl) | 17.69±18.70 | 6.13±2.55* | <0.001 |
| Osteopontin (ng/ml) | 24.73±19.12 | 12.23±10.54* | 0.021 |

*p<0.05, compared to PPROM Group.

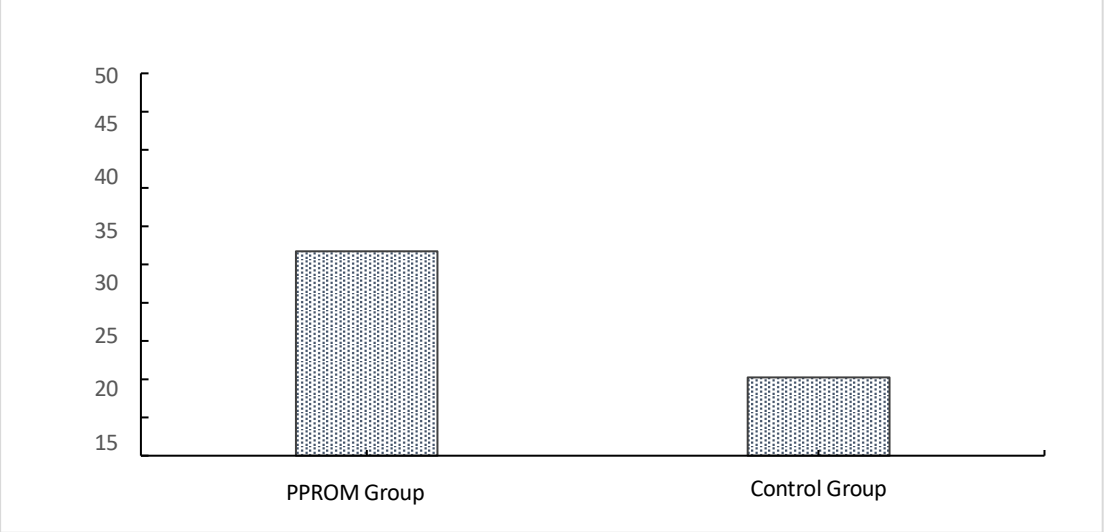


**Figure 1. Comparison of Osteopontin levels in groups.** (p<0.05 compared to Control Group)

## Discussion

PPROM is the most commonly associated cause of preterm labor[1]. PPROM is the rupture of fetal membranes before 37th gestational week. Various pathological conditions (sub-clinical or overt infection, inflammation, mechanical stress, bleeding etc.) might activate a cascade that cause PPROM. Even though many risk factors are mentioned, genital system infections are the most commonly defined risk factor for PPROM[1-3].

The management of PPROM is a much-debated topic because preterm labor is the most important cause of perinatal mortality and morbidity; many factors such as correct diagnosis, appropriate management and intervention, use of tocolytics, antibiotic prophylaxis and duration of administration, antenatal corticosteroid administration and timing, testing methods to be

used for infection, and the decision for delivery should be managed appropriately and at the right time[3]. In the early preterm fetus (< 34 weeks), prolonged gestation is associated with significant morbidity benefit, but PPROM-related complications (in utero infection, early placental detachment, cord prolapse/compression) should also be considered. At this point, a prediction correlated with the severity of the condition is needed and in the light of this information, we aimed to examine the relationship between PPROM and osteopontin and commonly used inflammation markers (CRP, white blood cell count, sedimentation) and to investigate the role of osteopontin in the prediction and appropriate management of PPROM. In the current study, serum Osteopontin and serum CRP, white blood cell and sedimentation values, which are frequently used inflammation markers, were found to be higher in patients diagnosed with PPROM compared to the control group. The study results support the previous studies regarding the role of serum osteopontin in inflammation[5,6].

In a cohort study conducted by Stephan M. et al[9]. on 386 pregnant women with PPROM, it is noted that C-reactive protein (CRP) was a significant marker for the identification of microbial infection of the amniotic cavity and histological chorio-amnionitis. Also, Balciuniene et al. demonstrated that CRP is the most reliable indicator of histological chorioamnionitis and can diagnose intrauterine infection earlier than WBC and ESR in a study of 80 pregnant women conducted in 2016[10]. Amirabi et al. examined the diagnostic values of WBC, CRP and ESR in 71 pregnant women with chorioamnionitis due to the premature rupture of membranes; and emphasized that these three markers may have a significant role in the diagnosis of PPROM[11]. However, ESR was reported to be less reliable among these markers[11]. In this present study, higher WBC, CRP and ESR levels were found to be statistically significant in the patient group, but ESR was less statistically significant.

Osteopontin was reported an important marker in inflammation, wound healing, granulomatous formations, fibrosis, regulation of nitric oxide, mineralization, dystrophic calcification, tumoral metastasis and protection of cell viability[5-8,12]. Osteopontin was proven to be expressed in functioning placental compartments in the study by Johnson et al.[13] Liu et al. demonstrated that co-synthesis of osteopontin and beta 3 integrin is a biological marker for implantation and has a crucial role in blastocyst implantation[14].

Osteopontin has been proven to play an important role for a successful pregnancy by Qu et al.[15] In this study conducted on mice, they showed that osteopontin was expressed in decidual natural killer cells and dNK; and osteopontin expression was significantly lower in patients with recurrent pregnancy loss . Winhofer et al. proved that osteopontin was positively correlated with progesterone, estrogen, liver enzymes and CRP concentrations[16]. In this current study, serum Osteopontin levels in patients diagnosed with PPROM were found to be statistically significantly higher than the control group.

This current study is the first in the literature to investigate serum Osteopontin levels in patients diagnosed with PPROM. However, including only 64 women, a relatively small number, only from a single hospital and defined geographical area are the limitations of our study, and prevent us to generalize our results for the whole society.

In conclusion, our study supports the hypothesis that serum Osteopontin levels increase in patients diagnosed with PPROM. Measuring serum Osteopontin levels may be used as an important marker in the prediction and

management of PPROM in the future. Large-scale studies including larger number of cases are required to support these results.

**Conflicts of Interest**

The authors have no conflicts of interest to declare.

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