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Original Research

Investigation of Fractalkine and MIP-1β Levels as Markers in Premature Membrane Rupture Cases: A Prospective Cohort Study

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Abstract

Background: To investigate fractalkine and MIP-1β levels in amniotic fluid and serum of cases with premature rupture of membranes. **Methods**: In this prospective cohort study, pregnant women who applied to the Firat University, Gynecology and Obstetrics Clinic with the diagnosis of premature rupture of membranes (PROM) between 24 weeks and 36^{+6} gestational weeks and who had elective cesarean section between 37–41 weeks of gestation were included. Amniotic fluid obtained during cesarean section and serum obtained from blood taken simultaneously from patients with cesarean section during sterile speculum examination in PROM cases were stored at –80 °C until the study day after the storage conditions were met. Tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interferon-gamma (IFN-γ), macrophage inflammatory protein-1 beta (MIP-1β) and fractalkine levels were measured by enzyme-linked immunosorbent assay (ELISA) from obtained serum and amniotic fluid samples. **Results**: There was no difference in age in both groups. Gravida, parity, gestational week, birth weight and umbilical artery pH values at birth were significantly higher in the control group than in the PROM group. Serum TNF-α, IL-1β, IFN-γ, MIP-1β and Fractalkine values were similar in both groups. However, while TNF-α values in amniotic fluid were similar in both groups, IL-1β, IFN-γ, MIP-1β and Fractalkine values were found to be significantly higher in the PROM group. **Conclusions**: Fractalkine and MIP-1β may be biomarkers worthy of investigation that can be used in the early diagnosis and prognosis of PROM cases.

Keywords: PROM; fractalkine; MIP-1β

1. Introduction

Premature rupture of membranes (PROM) is defined as spontaneous rupture of fetal membranes before 37 weeks of pregnancy [1] and complicates 5–7% of all pregnancies [2]. In addition to its spontaneous occurrence, it can also develop as a complication of invasive procedures such as amniocentesis, fetal surgery, cerclage placement. Mid-trimester preterm premature rupture of membranes (PPROM) develops before 28 weeks of gestation and is seen in approximately 0.4–0.7% of all pregnancies, and is associated with high neonatal mortality and severe short- and long-term morbidity [3]. Studies have reported that serum inflammatory marker levels are elevated in PROM [4,5] and perinatal mortality is associated with gestational week at birth, birth weight, and presence of fetal growth restriction and anhydramnios [6,7].

It has been shown that the matrix metalloproteinase-9 (MMP-9) concentrations increase in the membrane rupture mechanism in fetuses with PROM, but interleukin-1 beta (IL-1β), soluble tumor necrosis factor receptors 1 (sTNF-R1) and sTNF-R2 concentrations are lower than in fetuses with preterm labor and intact membranes [8]. Bacterial products, proinflammatory cytokines, IL-1β and tumor necrosis factor-alpha (TNF-α) can induce expression of metalloproteinases such as MMP-9 in cases with chorioamnionitis [9].

Chemokine ligand 1 (CX3CL1) is the only known representative of the δ-chemokine family. CX3CL1 is now called fractalkine [10]. Interleukin-2 (IL-2) induces cluster of differentiation 4+ (CD4+) and CD8+ T cells, leading to upregulation of fractalkine receptor-1 (CX3CR1) expression. The proinflammatory cytokines interferon-gamma (IFN-γ), TNF-α and IL-1β activate fractalkine expression in blood vessels [11–14]. Because of these properties, fractalkine plays an important role in the immune response of immune system cells [15]. In addition, CX3CL1's chemoattractant and adhesive properties that nurture inflammatory and angiogenesis processes are upregulated by hypoxic conditions and inflammatory conditions, particularly diabetic placenta [16].

Fractalkine, together with other cytokines such as chemokine CC motif ligand 7 (CCL7), CCL4 (MIP-1β), CCL14, is involved in the implantation process, placental angiogenesis, invasion of trophoblasts into spiral arteries,

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response to inflammatory and immunological factors at the uterus-placental interface, and initiation of labor [17,18].

In recent years, various potential biomarkers such as placental protein 14 in amniotic fluid and high-mobility box-1 have been investigated for use in the clinical diagnosis of PROM [19,20]. An ideal test should be non-invasive, fast, accurate, cost-effective, easily applicable and easily accessible [21].

Currently, analyzes of amniotic fluid (AF) samples obtained through amniocentesis are generally considered the gold standard approach to identify microbial invasion of the amniotic cavity (MIAC)/intraamniotic infection (IAI) in PROM. In particular, IL-6, IL-8 and matrix metalloproteinase (MMP)-8 and -9 in AF have been shown to be the strongest markers of intra-amniotic infectious and inflammatory states in PROM [22]. However, their measurements in current practice may be of limited use due to their invasiveness (e.g., amniocentesis) and relatively low sensitivity [23]. Although assessment of maternal blood markers may serve as a non-invasive, desirable and inexpensive approach for the anticipated management of women with PROM, these inflammatory biomarkers have not been adequately studied in plasma samples from women with PPROM in relation to MIAC/IAI [22]. In our single-center, prospective cohort study, we aimed to investigate whether fractalkin and MIP-1β could be a non-invasive biomarker in the diagnosis of PROM and perhaps in predicting PROM.

2. Materials and Methods

This prospective cohort study was carried out on 80 patients who applied to the Firat University, Faculty of Medicine, Gynecology and Obstetrics Clinic within the period of 1 January 2022 to 1 September 2022 after obtaining the approval of the Firat University Clinical Ethics Committee (number 2021-13-46). The patients were divided into 2 groups. We selected 40 patients with the required inclusion criteria during the time period for each group who met the study criteria.

Group 1: 40 healthy pregnant patients without PROM who were pregnant between 37 and 41 weeks and had cesarean section due to elective reasons.

Group 2: 40 pregnant patients who had PROM 24 hours before delivery and were pregnant between 24 weeks and 36^{+6} weeks and delivered by cesarean section.

The patients were questioned in terms of age, parity, drug use, and whether they had a systemic disease.

2.1 Exclusion Conditions

Pregnant women with preterm birth, pregnant women younger than 24 weeks of gestation, hypertensive pregnant women, acute or chronic infections, pregnant women with preeclampsia or eclampsia, gestational diabetes, and intrauterine growth retardation.

Pregnant women who applied to the clinic with the pre-diagnosis of PROM were examined with a sterile speculum (REF T201793, TMS Medikal ve plastik ürünler san. dış. tic. ltd., Istanbul, Turkey). About 5 mL of amniotic fluid accumulated in the lower spoon of the speculum from the cases with active amniotic fluid presentation was drawn into the injector, put into Eppendorf tubes, and quickly stored in a deep freezer at –80 °C until the working day. In the meantime, the diagnosis of PROM was confirmed by performing placental alpha microglobulin-1 protein test (AmniSure test). At the same time, approximately 5 mL of venous blood was collected from the patients, centrifuged and stored at –80 °C until the study day. All PROM cases were followed up in the hospital until the pregnancy was terminated. In the follow-up, fever, heart rate and blood pressure were monitored. At daily visits, foul-smelling vaginal discharge was investigated and the presence of fundal tenderness was checked with fundal examination. Weekly sedimentation, leukocyte, C-reactive protein (CRP) and procalcitonin values were measured. In all cases under 34 weeks of age, 2 doses of betamethasone (Celestone Chronodose, Schering-Eczacıbaşı, Lüleburgaz, Turkey), 24 mg in total, were administered intramuscularly at 24-hour intervals for lung maturation. Ampicillin (Ampisina® 1 gr Flakon, Mustafa Nevzat Pharmaceuticals, Istanbul, Turkey) treatment was started in all cases and continued until delivery. All pregnant women who were diagnosed with PROM under 34 weeks of gestation were followed up spontaneously, with necessary medication and follow-up. In PROM cases, amniotic fluid and maternal serum samples were obtained during the first examination and stored.

Pregnancies that reached 34th gestational week were delivered according to the indication (normal labor induction or cesarean section delivery) and were followed up in the clinic for 2 more days postoperatively. Pregnancies admitted with the diagnosis of PROM between 34–37 weeks of gestation were hospitalized and their pregnancies were terminated according to the indication, and blood and amniotic fluid samples were collected and stored as described above.

Pregnant women who applied to the clinic between 37–41 weeks of gestation and met the exclusion criteria listed above and would have elective cesarean section constituted the control group. Moreover, 5 mL of blood and about 5 mL of sterile amniotic fluid were collected from these pregnant women during cesarean section, and then stored at –80 °C until the study day. TNF-α, IL-1β, IFN-γ, macrophage inflammatory protein-1 beta (MIP-1β) and fractalkine levels were measured in serum and amniotic fluid by enzyme-linked immunosorbent assay (ELISA) method.

2.2 Biochemical Measurements

Biochemical measurements were made using human ELISA kits in serum and amniotic fluid in accordance with the relevant kit procedures. Obtained absorbances were

Table 1. Dermographic parameters of group 1 (control) and group 2 (PROM).

Parameters	Group 1 (Control) (n = 40)		Group 2 (PROM) (n = 40)		*p* values
	Median (min–max)	25%–75% percentile	Median (min–max)	25%–75% percentile	
Age (years)	31 (24–41)	27.25–34.75	29 (20–41)	26–36	0.434
Gestational week	38 (37–41)	37.25–38	34 (24–36)	32–35	<0.001*
Gravida	4 (1–10)	2–5.75	2 (1–9)	1–3.75	0.005*
Parity	2 (0–6)	1–3.75	1 (0–4)	0–2	0.010*
Birth weight (g)	3000 (2400–3960)	2900–3337	2275 (620–3140)	1600–2625	<0.001*
pH value	7.36 (7.31– 7.44)	7.33–7.37	7.33 (7.22–7.48)	7.30–7.37	0.032*

* = Compared with Group 1. PROM, premature rupture of membranes.

Group 1 = Control group; Group 2 = PROM (premature rupture of membranes) group. Values are presented as median (min–max) and 25%–75% percentile, $p < 0.05$ was considered to be statistically significant.

read spectrophotometrically at 450 nm in an EPOCH 2 (Bio Tek Instrument, Inc, Winoosky, VT, USA) microplate reader and the results were obtained.

TNF-α (Sun red Biotechnology Company, Shanghai, China; catalog no = 201-12-0083; kit measuring range = 3–900 ng/L; kit sensitivity = 2827 ng/L);

IL-1β (Sun red Biotechnology Company, Shanghai, China; catalog no = 201-12-0144; kit measuring range = 200–800 pg/mL; kit sensitivity = 15,013 pg/mL);

IFN-γ (Sun red Biotechnology Company, Shanghai, China; catalog no = 201-12-0106; kit measuring range = 2–600 ng/L; kit sensitivity = 1706 ng/L);

MIP-1β (Sun red Biotechnology Company, Shanghai, China; catalog no = 201-12-0088; kit measuring range = 0.5–150 pg/mL; kit sensitivity = 0.432 pg/mL);

Fractalkine (CX3CL1) (Sun red Biotechnology Company, Shanghai, China; catalog no = 201-12-2102; kit measuring range = 0.2–30 ng/mL; kit sensitivity = 0.102 ng/mL).

2.3 Statistical Analysis

SPSS v.21.0 (IBM Corporation, Armonk, NY, USA) package program was used for statistical analysis of the data. In data analysis, the distribution of continuous variables was determined by Shapiro-Wilk normality tests. Data that did not fit the normal distribution were expressed as median (IQR: interquartile range) values, while qualitative data were expressed as percentage values. Two independent groups were compared with the Mann-Whitney U test. $p < 0.05$ was considered to be statistically significant.

3. Results

Age was similar between the groups. Gravida, parity, gestational week, birth weight and umbilical cord pH values in the control group compared to the PROM group was significantly higher (Table 1).

Serum TNF-α, IL-1β, IFN-γ, MIP-1β and fractalkine values were similar in both groups (Table 2).

While amniotic fluid TNF-α values were similar in both groups, IL-1β, IFN-γ, MIP-1β and fractalkine values were significantly higher in the PROM group (Table 3). However, we can think that the confidence interval (CI) is large due to the small number of subjects. Therefore, studies with a larger number of cases are needed.

4. Discussion

As a result of our study, we showed that there was a significant decrease in birth weight of PROM cases because their pregnancies were terminated earlier. In addition, although the umbilical artery pH value at birth was within clinically normal limits, it was significantly lower in the PROM group. We detected a significant increase in the levels of proinflammatory cytokines Fractalkine and MIP-1β in the amniotic fluid in patients with PROM compared to the control group. We also found a significant increase in IL-1β and IFN-γ levels in amniotic fluid. However, we could not detect any difference in serum levels in both groups. While inflammatory and proinflammatory cytokine levels in the amniotic fluid can be increased in PROM cases, the similarity of serum levels supports an inflammatory homeostasis in pregnancy. We think that there may be a strong relationship between the pathophysiology of PROM and fractalkine and MIP-1β.

In our study, we found that gravida and parity were significantly lower in our PROM group. The fact that the gestational week and birth weights were also lower in the PROM group is due to the fact that we delivered our PROM cases around the 34th week.

Chemokines [24] act as chemo-attractants that modulate the migration of leukocytes and the immune response [25]. The major roles of membrane-bound CX3CL1 are by activating target cells, promoting leukocyte attachment and adhesion. In humans, fractalkine is chemotactic for T cells and monocytes [26]. Many stimuli that can affect cell homeostasis potentially induce fractalkine secretion [27]. Fractalkine also exerts its effects through the fractalkine receptor (CX3CR1) [10]. It has been shown that maternal plasma cytokines (IL-1β, IL-2, IL-6, IFN-γ and TNF-α) concentrations do not differ significantly between patients with clinical chorioamnionitis (intra-amniotic inflam-

Table 2. TNF-α, IL-1β, IFN-γ, MIP-1β, Fractalkine (CX3CL1) levels in maternal serum.

Parameters	Group 1 (Control) (n = 40)		Group 2 (PROM) (n = 40)		*p* values
	Median (min–max)	25%–75% percentile	Median (min–max)	25%–75% percentile	
TNF-α, ng/L	113.6 (7.50–18511)	55.5050–171.7	108 (7.5–970.55)	53–148.66	0.840
IL-1β, pg/mL	1180.51 (75–634543)	632–3003	909 (29.27–9824)	332–1531.4	0.138
IFN-γ, ng/L	106.28 (1.89–308.91)	80.3925–177.7	107.9 (56.7–629)	71.4–186.5	0.744
MIP-1β, pg/mL	25.54 (1.24–25532)	17.3–55.7	25 (13.68–51013)	22.6–33.7	0.400
Fractalkine (CX3CL1), ng/mL	0.29 (0.25–37)	0.25–8.27	2.88 (0.24–94.5)	2.12–4.96	0.227

Group 1 = Control group; Group 2 = PROM (premature rupture of membranes) group; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin-1 beta; IFN-γ, interferon-gamma; MIP-1β, macrophage inflammatory protein-1 beta; CX3CL1, chemokine ligand 1. Values are presented as median (min–max) and 25%–75% percentile, $p < 0.05$ was considered to be statistically significant.

Table 3. TNF-α, IL-1β, IFN-γ, MIP-1β, Fractalkine (CX3CL1) levels in amniotic fluid, values are presented as median (min–max) and 25%–75% percentile, $p < 0.05$ was considered to be statistically significant.

Parameters	Group 1 (Control) (n = 40)		Group 2 (PROM) (n = 40)		*p* values
	Median (min–max)	25%–75% percentile	Median (min–max)	25%–75% percentile	
TNF-α, ng/L	83.74 (19.77–9239)	38.8250–96.9	54.16 (7.5–159088)	12.21–154.6475	0.893
IL-1β, pg/mL	625.58 (32.99–1301.2)	296–1160	1558.79 (75–2813,58)	895–2262	<0.001*
IFN-γ, ng/L	22.9 (15.99–68.12)	21.33–26.72	79.915 (16.07–192.1)	44.4–129.33	<0.001*
MIP-1β, pg/mL	11.43 (5.73–23.92)	6.215–15.21	23.125 (1.47–57.7)	10.335–38.68	<0.001*
Fractalkine (CX3CL1), ng/mL	0.25 (0.25–2.99)	0.25–1.81	3.84 (0.25–14.63)	1.4925–7.155	<0.001*

Group 1 = Control group; Group 2 = PROM (premature rupture of membranes) group; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin-1 beta; IFN-γ, interferon-gamma; MIP-1β, macrophage inflammatory protein-1 beta; CX3CL1, chemokine ligand 1.
* = Compared with Group 1.

mation with and without detectable bacteria and without intra-amniotic inflammation). Therefore, it was stated that maternal plasma cytokine concentrations would have limited value in identifying patients with bacteria in the amniotic cavity. It was concluded that for an accurate assessment of the presence of amniotic infection, amniotic fluid analysis would be required [28]. In our study, we saw that while serum TNF-α, IL-1β, IFN-γ, MIP-1β and fractalkine levels were similar before clinical PROM developed in the control group and our PROM group, there was a significant increase in IL-1β, IFN-γ, MIP-1β and fractalkine levels in the amniotic fluid in the PROM group, except for TNF-α.

Generally, chemokines are involved in immune system homeostasis, while inducible chemokines are primarily involved in inflammatory processes. Chemokines are divided into four subfamilies according to the number and spacing of the first two cysteine residues [29]. These four subfamilies are called C, CC, CXC, and CX3C. Here C represents a cysteine and X represents an amino acid residue. It has been shown that the level of fractalkine increases in the first trimester of pregnancy and in term pregnancy complicated by preeclampsia [30–32]. We also included cases with PROM starting from the 3rd trimester in our study. In addition, we excluded complicated pregnancies such as diabetes, intrauterine growth retardation, hypertension and preeclampsia. Thus, we aimed to prevent the inflammatory cytokine and fractalkine levels in the blood and amniotic fluid from being affected by any other complicated condition. In our control group, we included the cases without complicated pregnancy and at 37 and 41 weeks of gestation, because we thought that fractalkine and other inflammatory cytokines may play a role in the pathophysiology of preterm birth. It could be considered that the control group should be selected from patients with cord prolapse or those who underwent amniocentesis. We did not choose these groups as the control group, because cord prolapse results in PROM and membrane damage. A similar situation may occur in amniocentesis and amniocentesis is usually performed before the third trimester. For these reasons, and also because the third trimester amniocentesis and cord prolapse are very few, we selected our control group from cesarean section cases between 37–41 weeks.

Fractalkine regulates adhesion and migration in fetal-maternal interaction at different stages of human pregnancy. However, some pregnancy pathologies such as chorioamnionitis [33] and severe early-onset preeclampsia [34] have been suggested to be associated with increased placental fractalkine expression. Hannan *et al.* [35] reported that serum fractalkine levels were higher in patients who had abortion in the first trimester, while it was undetectable in patients who had normal delivery. Li *et al.* [36] showed that fractalkine and fractalkine receptors were significantly upregulated in the uterus of IFN-γ-induced abortion mice. It has been shown that fractalkine plays a role in

the feto-maternal interaction in the placental invasion process and may be associated with missed abortion [34,35]. Therefore, we created both PROM and control groups from uncomplicated pregnancies in the third trimester. We tried to prevent the parameters that we evaluated in this way from being affected by possible complications.

Chemokine CC motif ligand 4 (CCL4) (MIP-1) has been shown to be involved in implantation [37]. However, high serum MIP-1β values have been associated with the presence of active infections during pregnancy [38]. In our study, we observed that MIP-1β levels in the amniotic fluid were significantly increased in the PROM group before clinical signs of infection developed compared to the control group. We followed all our PROM cases in the clinic. We terminated the pregnancy at 34 weeks. Therefore, we thought that we could not find a difference in serum MIP-1β and other inflammatory markers, since we terminated the pregnancy. We also excluded the cases who were given betamethasone for lung maturation and gave birth within 48 hours.

Based on the study, it is accepted that normal pregnancy is actually a controlled inflammatory state and many pregnancy-related complications are associated with an exaggerated local or systemic inflammatory response [39]. Therefore, it has been reported that a successful pregnancy depends on the balance between anti-inflammatory and pro-inflammatory cytokines [40]. MIP-1β and fractalkine along with some other cytokines are involved in the response to inflammatory and immunological factors in implantation, invasion of trophoblast into spiral uterine arteries, processes of placental angiogenesis, and at the uterus-placental interface during delivery [17,18]. In our study, we evaluated MIP-1β levels together with fractalkine. In our study we didn't find any significant difference in TNF-α, IL-1β, IFN-γ, MIP-1β serum levels between groups but there was a significant increase in IL-1β, IFN-γ, MIP-1β, except TNF-α in amniotic fluid of PROM cases. This may show that pregnancy is a controlled inflammatory state and that there is a homeostasis.

Human amniotic epithelial cells (HAEC) are released into the amniotic fluid by various cytokines such as chemokines and fractalkine, and the chemokine profile of HAEC during pregnancy can be altered by many factors. Up-regulation of this chemokine expression by inflammatory stimuli has been considered, since fractalkine provides regulation of lymphocyte accumulation at sites of inflammation [41,42]. However, inflammation is often accompanied by significant local decreases in oxygen availability [43] and hypoxia has been shown to significantly inhibit fractalkine production [13,44]. This is a confusing situation. Because both inflammation and hypoxia cause a local increase of TNF-α and is a potent inducer of fractalkine production and fractalkine receptor expression in TNF-α [45,46]. In our study, TNF-α [47], a proinflammatory cytokine, was similar in amniotic fluid in both groups. However, fractalkine levels were increased in our PROM group. We can explain this situation with the following theory: TNF-α may have increased fractalkine production and this increase may have suppressed TNF-α production with a negative feedback mechanism. In this way, an inflammatory homeostasis can be achieved by preventing an excessive inflammatory response. In addition, as a result of our study, we found that fetal umbilical cord pH values were significantly lower in our PROM group. On the contrary, as mentioned above, we found high fractalkine and MIP-1β levels in amniotic fluid. We interpreted this situation as confusing. This situation could be explained to some extent by measuring the pH values of the amniotic fluid and the placenta. However, we did not look at the pH values of the amniotic fluid and placenta.

Chorioamnionitis is inflammation of the amniotic and chorionic membranes and is associated with significant maternal and perinatal adverse outcomes [48]. Clinical chorioamnionitis is characterized by maternal fever, leukocytosis, tachycardia, uterine tenderness and premature rupture of membranes (PROM). Subclinical/histological chorioamnionitis, which is more common than clinical chorioamnionitis, is asymptomatic and is characterized by inflammation of the chorion, amnion, and placenta [49]. The frequency of histologic chorioamnionitis is higher than clinical chorioamnionitis with positive bacterial cultures. Since the incidence of histological chorioamnionitis in PROM is up to 50%, pathological evaluation of the placenta is required for a definitive diagnosis [50]. The role of fractalkine in premature rupture of membranes (PROM) and preterm labor is still under investigation [51]. None of our cases had clinical chorioamnionitis during the study. Therefore, we could not evaluate the proinflammatory and inflammatory cytokine levels in PROM and chorioamnionitis and make a comment.

One study showed that none of the proteins measured in maternal plasma were associated with IAI. In addition, cytokine/chemokine levels in plasma were reported to be weakly correlated with those in amniotic fluid (AF) in this study. These findings also indicate that microbes or microbe-derived cytokines/chemokines in the amniotic cavity are poorly reflected in plasma. Therefore, it is very difficult to identify new non-invasive biomarkers for IAI and/or to perform MIAC using maternal blood samples, especially in women with preterm labor (PTL) [52]. Evaluation of protein levels using maternal blood samples has been shown in one study to have limited clinical value for the noninvasive identification of PROM pregnancies complicated by MIAC/IAI [22]. As a result of our study, we showed that cytokine/chemokine levels were significantly increased in amniotic fluid rather than maternal serum in PROM cases. This shows that amniotic fluid is more valuable in determining PROM cases.

Limitations of our study: (1) Limited number of cases, our parameters could not be studied to compare the 1st, 2nd

and 3rd trimester PROM cases of the study. (2) Our study was not grouped according to PROM durations. (3) The absence of a clinical chorioamnionitis group in our study is another limitation. Since chorioamnionitis did not develop clinically in any of our cases during the study, we could not form the chorioamnionitis group. (4) Another limitation of ours is the inability to examine the placenta histologically and examine the presence of inflammation, and the parameters we studied were not evaluated both biochemically and immunohistochemically.

The strengths of our study are that it is a prospective cohort study, and fractalkine levels in maternal serum and amniotic fluid have been studied for the first time. With further studies, the potential of fractalkin levels to be an early biomarker has been emphasized in the early diagnosis of PROM and the determination of cases at risk of developing PROM.

5. Conclusions

In conclusion, fractalkine and MIP-1β may be biomarkers worthy of investigation that can be used in the early diagnosis and prognosis of PROM cases.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Author Contributions

ŞP and RA designed the study. ŞP, BÇ, MKA, MDC performed the research, collected data. SCO analyzed the data, helped and advised on writing manuscript. ŞP and RA wrote manuscript. Nİ gave the results of study. MY analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was carried out at Fırat University, Faculty of Medicine, Gynecology and Obstetrics Clinic after obtaining the approval of the Fırat University Clinical Ethics Committee number 2021-13-46. All patient's informed consent is obtained.

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Conflict of Interest

The authors declare no conflict of interest. Süleyman Cemil Oğlak is serving as one of the Guest editors of this journal. We declare that Süleyman Cemil Oğlak had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Michael H. Dahan.

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