

## Research Article

## C-Reactive Protein and Matrix Metalloproteinase-7 in Preterm Premature Rupture of Membranes (PPROM) and Premature Rupture of Membranes (PROM)

### *C-Reactive Protein dan Matrix Metalloproteinase-7 pada Ketuban Pecah Dini Kehamilan Preterm dan Aterm*

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#### Abstract

**Objective:** To determine the difference in qualitative serum CRP and MMP-7 levels between PROM and PPRM.

**Methods:** Research with a case-control design with premature rupture of membranes in term pregnancy as a control group and preterm pregnancy as a case group conducted in the delivery room of the Regional General Hospital dr. Zainoel Abidin Banda Aceh starting from January 2020. Total number of each group as many as 30 samples that met the inclusion and exclusion criteria by taking venous blood samples to check the levels of qualitative CRP serum and levels of the Metalloproteinase-7 Matrix (MMP-7). Statistical tests for categorical - numerical data groups were tested using *independent sample t-test* and categorical - categorical data groups were tested using Chi-Square test.

**Results:** A total of 60 samples with 30 PPRM and 30 PROM mothers. There was no statistically significant difference in the qualitative CRP serum result in PROM patients with term and preterm pregnancies. On MMP-7 examination, the mean MMP-7 level of preterm PROM mothers was higher than at term PROM, which was 5.28 mg / L and there was a significant relationship between MMP-7 and qualitative CRP in PROM patients with term pregnancy ( $p < 0.05$ ).

**Conclusion:** There is no significant difference in the value of qualitative CRP and MMP-7 between PPRM and PROM. However, there was a significant association between qualitative serum CRP and plasma MMP-7 in PROM.

**Keywords:** c-reactive protein, matrix metalloproteinase 7, rupture of membranes.

#### Abstrak

**Tujuan:** Untuk mengetahui perbedaan gambaran serum CRP kualitatif dan kadar MMP-7 antara kehamilan preterm dan aterm dengan ketuban pecah dini di RSUD Dr. Zainoel Abidin Banda Aceh.

**Metode:** Penelitian dengan desain kasus kontrol dengan ketuban pecah dini kehamilan aterm sebagai kelompok kontrol dan kehamilan preterm sebagai kelompok kasus. yang dilakukan di Kamar Bersalin Rumah Sakit Umum Daerah dr. Zainoel Abidin Banda Aceh pada Januari 2020. Dengan jumlah masing-masing kelompok sebanyak 30 sampel yang memenuhi kriteria inklusi dan eklusi dengan pengambilan sampel darah vena untuk dilakukan pemeriksaan kadar serum CRP kualitatif dan kadar Matrix Metalloproteinase-7 (MMP-7). Uji statistik untuk kelompok data yang bersifat kategorik - numerik diuji menggunakan uji T tidak berpasangan dan pasangan kelompok data yang bersifat kategorik - kategorik akan diuji menggunakan uji Chi-Square.

**Hasil:** Sebanyak 60 penderita KPD dengan 30 Ibu KPD Preterm dan 30 KPD aterm. Tidak dijumpai perbedaan yang bermakna secara statistik gambaran serum CRP kualitatif pada penderita KPD dengan kehamilan aterm dan preterm. Pada pemeriksaan MMP-7 rerata kadar MMP-7 ibu KPD preterm lebih tinggi dibandingkan KPD aterm yakni senilai 5,28 mg/L dan terdapat hubungan bermakna antara MMP-7 dan CRP kualitatif pada penderita KPD dengan kehamilan aterm ( $p < 0,05$ ).

**Kesimpulan:** Tidak terdapat perbedaan gambaran signifikan nilai CRP kualitatif dan MMP-7 antara KPD preterm dan aterm. Namun, terdapat hubungan yang signifikan antara serum CRP kualitatif dan MMP-7 plasma pada penderita ketuban pecah dini dengan kehamilan aterm.

**Kata kunci:** c-reactive protein, matrix metalloproteinase-7, ketuban pecah dini.

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## INTRODUCTION

Preterm premature rupture of membranes (PPROM) is defined as rupture of the membranes that occur before labour signs under 37 weeks of gestation while Premature rupture of membranes (PROM) takes place at 37 weeks of gestation or later.<sup>1-3</sup> The incidence of PROM is a global obstetric problem with the incidence rate varying between 5-10% of all births. In term pregnancy, as many as 70% and 30% of PROM cases cause preterm birth. The incidence of PROM also plays a role in causing chorioamnionitis infection which can lead to sepsis, therefore increasing maternal and perinatal morbidity and mortality due to ascending infection and cord compression.<sup>4-6</sup>

Various risk factors are suspected to be the basis for the occurrence of PROM. The incidence of PROM in preterm pregnancy can also be caused by infection or inflammation of the choriodecidua and a decrease in the amount of collagen from the amniotic membrane is thought to be a predisposing factor for preterm PROM.<sup>7</sup> Early identification of infection due to PROM is important to prevent complications. However, the results of the parameters for the examination of infection in the basic laboratory such as blood leukocyte count, sedimentation rate, and specificity are difficult to rely on because of their low sensitivity and specificity due to physiological and hematologic changes in pregnant women.<sup>8</sup>

One of the parameters for establishing an early diagnosis or a useful marker of an infection and inflammation process is C-Reactive Protein (CRP) which is an acute-phase protein that can be an early and reliable indicator of clinical histopathology and chorioamnionitis.<sup>8,9</sup> Several studies have shown that the mechanical strength of fetal membranes and reduced tension resistance in PROM due to increased collagen degradation and matrix metalloproteinase (MMP) are the main mediators of collagen degradation from the amniotic membrane.<sup>10,11</sup> Multiple MMPs participate in the degradation of the extracellular matrix of the fetal membrane at preterm or in labor. Metalloproteinase-7 (MMP-7) or matrilysin is classified as collagenase which can cause degradation of the amniotic membrane and can decrease various components of the matrix including collagen type IV, fibronectin, elastin, and laminin.<sup>10,12</sup>

Several studies have assessed the increase in both CRP and MMP in premature rupture of membranes. However, differences in their value

between preterm and term pregnancies is not yet known. The authors of this study wanted to know the differences in serum levels of CRP and MMP-7 associated with PPRM and PROM cases at the Regional General Hospital Dr. Zainoel Abidin Banda Aceh.

## METHODS

This was an unpaired numerical comparative analytic study with a case-control design conducted in the Maternity Room of the Regional General Hospital dr. Zainoel Abidin Banda Aceh starting from January 2020 until the amount of sample is fulfilled. The sample of this study is population that meets the research criteria in the form of inclusion criteria, namely PPRM pregnant women, PROM pregnant women, single fetuses living intrauterine, and those who are willing to participate by signing informed consent form. Exclusion criteria includes pregnancy with inflammatory diseases and systemic infectious diseases such as pneumonia, typhoid, malaria, hepatitis, pre-eclampsia, eclampsia, antepartum hemorrhage, prior antibiotic treatment, cervical incompetence, polyhydramnios, history of trauma during pregnancy, mothers with smoking habits, multiple pregnancies, and fetuses with congenital abnormalities.

The results of the sample calculation for preterm and term group were 30 respondents. The research procedure was carried out by examining all pregnant women with PROM who came to the delivery room of the Regional General Hospital dr. Zainoel Abidin Banda Aceh, who was included in the inclusion criteria. For each patient, history taking was done followed by ultrasound examination to rule out the exclusion criteria. Examination of the amniotic fluid either by direct observation, examination with inspeculo or with nitrazine paper then informed consent was then carried out. 6 cc of blood samples were taken from 6 thecubital veins to check qualitative CRP serum and MMP-7 levels, then the samples were centrifuged at a speed of 40,000 rpm for 10 minutes until the serum was divided into 10 small serum tubes of 0.3 cc each and stored in a refrigerator with a temperature of -40° C and was sent to Prodia Laboratory for further examination.

The data obtained from the research will be analyzed in univariate and bivariate methods. Categorical and numerical group pairs were tested using the independent sample t-test and samples that did not meet the requirements of

the normal distribution based on the Kolmogorov Smirnov test were tested using the Mann Whitney U test. Pairs of categorical and categorical data groups were tested using the Chi-Square test with the significance level used was  $\alpha = 0.05$  with 95% Confidence Interval.

## RESULTS

After the data was collected, there were 60 patients with rupture of the membranes with 30 mothers with gestation <37 weeks classified as preterm pregnancy (case group) and 30 mothers with gestation  $\geq 37$  weeks classified as term pregnancy (control group).

Based on the characteristics of our research subjects including age and obstetric status, the age of PROM patients were relatively younger than PPRM patients. However, there is no significant difference in the mean age between the two groups ( $p > 0.05$ ). From their obstetrical status, both groups of PROM patients with term and preterm pregnancies showed the same distribution, namely Gravida 2. From their obstetric status, we did not find any significant difference between the two groups with a significance value of  $> 0.05$ .

Analysis of differences in qualitative CRP serum images in PROM patients with term and preterm pregnancies is presented in Table 1.

**Table 1.** Analysis of Differences in Qualitative Serum CRP Features in PROM and PPRM

|         | Rupture of membranes |       | P-value* |
|---------|----------------------|-------|----------|
|         | Preterm              | Aterm |          |
| CRP (+) | 7                    | 6     | 0.754    |
| CRP (-) | 23                   | 24    |          |

\* Chi-Squared test

According to the Chi-squared test in Table 1. there were 7 PROM mothers with preterm pregnancy who showed positive CRP values. Statistically, the qualitative CRP serum appearance on PROM and PPRM did not show any significant difference.

Analysis of differences in levels of MMP-7 in PROM and PPRM patients is presented in Table 2.

**Table 2.** Analysis of Differences in Levels of MMP-7 in PROM and PPRM

| Rupture of membranes Group | Median | Min - Max   | P-value* |
|----------------------------|--------|-------------|----------|
| Preterm pregnancy, n = 30  | 5.28   | 1.95 – 11.7 | 0.109    |
| Aterm pregnancy, n = 30    | 4.34   | 2.7 – 10.37 |          |

\* Mann-Whitney U test

Table 2. analyzes the differences in levels of MMP-7 in PROM and PPRM. The average MMP-7 level of PROM women with preterm pregnancy was higher than that of term pregnancy, which was 5.28 mg / L. Based on statistical tests using the Mann-Whitney U test, it is known that there is no significant difference in levels of MMP-7 in PROM and PPRM patients.

Analysis of the relationship between plasma MMP-7 levels and qualitative CRP in PROM patients is presented in Table 3.

**Table 3.** Analysis of the Relationship between Plasma MMP-7 Levels and Qualitative CRP in PROM

| CRP             | Median | Min - Max   | P-value* |
|-----------------|--------|-------------|----------|
| Positive, n = 6 | 3.4    | 2.76 – 4.36 | 0.015    |
| Negative n = 24 | 4.6    | 2.7 – 10.37 |          |

\* Mann-Whitney U test

Table 3. analyzes the relationship between plasma MMP-7 levels and qualitative CRP at PROM and PPRM. Patients with a negative qualitative CRP value had higher plasma MMP-7 levels than positive CRP, with a mean level of 4.6 mg / L. Statistically, based on the Mann-Whitney U test, it was found that there was a significant relationship between MMP-7 and qualitative CRP in PROM patients ( $p < 0.05$ ).

**Table 4.** Analysis of the Relationship between Plasma MMP-7 Levels and Qualitative CRP in PPRM

| CRP              | Mean $\pm$ SD   | P-value |
|------------------|-----------------|---------|
| Positive, n = 7  | 4.58 $\pm$ 1.65 | 0.130   |
| Negative, n = 23 | 6.03 $\pm$ 2.26 |         |

\* Independent sample t-test

Analysis of the relationship between plasma MMP-7 levels and qualitative CRP in PPRM patients is presented in table 4. Patients with negative qualitative CRP values had higher levels of MMP-7 than positive CRP with a mean difference of 1.45 mg / L. Statistically, based on the Independent sample t-test, it is known that there is no significant relationship between MMP-7 and qualitative CRP in PPRM patients ( $p > 0.05$ ).

## DISCUSSION

In theory, an infection can cause PROM through several mechanisms. Macrophages and other cells also produce and release various cytokines such as IL-1 which is an endogenous pyrogen, TNF- $\alpha$ , and IL-6 when infection occurs. These three cytokines stimulate the liver to synthesize and release several plasma proteins such as acute-phase proteins, including CRP which can increase rapidly.<sup>13</sup> The synthesis of CRP in the liver takes place very rapidly with serum concentrations increasing above 5mg / L for 6-8 hours and reaching optimum levels in around 24-48 hours. CRP levels will drop dramatically when the inflammatory process or tissue damage subsides and within about 24-48 hours it reaches normal values again. CRP levels will be stable in plasma and are not influenced by diurnal variations.<sup>13,14</sup>

In this study, statistically, the analysis of differences in the qualitative features of CRP serum in PROM and PPRM patients using the Chi-squared test did not show any significant differences. Several previous studies compared CRP values between PROM and normal pregnancy, some analyzed CRP quantitatively and obtained similar results, namely that there were significant differences between the control group and the case group, thus showing that CRP was very well used as an early predictor of subclinical chorioamnionitis. In contrast to this study, when one examines a conclusion that the CRP value will indeed increase significantly in cases of PPRM and PROM.<sup>9,13,15</sup> However, if the description of the CRP value between the two groups is compared, it is also not a qualitatively significant difference.

Research has shown that collagen degradation can also occur in an intracellular pathway. Most have focused on the role of MMP-2 and MMP-9 in the rupture of the amniotic membrane, and only a few are concerned about other members of the MMP group (such as MMP-7) which have

high degradative activity properties and are capable of triggering the cascade activation of other members of the MMP group.<sup>16,17</sup> Nishihara et al. found increased expression and activity of MMP-7 in amnion in PROM cases. These data suggest that MMP-7 may play an important role in rupture of the membrane and precipitate preterm birth.<sup>18</sup>

MMP-7 levels in PPRM patients in this study ranged from 1.95 - 11.7 mg / L. While the levels of MMP-7 in PROM mothers ranged from 2.7 - 10.37 mg / L. The mean plasma MMP-7 levels of PROM women with preterm pregnancy were higher than those of term pregnancies, namely 5.28 mg / L. This is different from that described by Wang et al., That the amniotic fluid concentration of MMP-7 increased significantly with increasing gestational age (Spearman  $\rho$ ,  $r = 0.8$ ;  $P < .001$ ).<sup>(17)</sup> A previous study concluded that intra-amniotic infection is associated with a significant increase in Matrilysin (MMP-7) in the amniotic fluid in preterm labor and patients with PPRM without microbial invasion of the amniotic cavity. The MMP-7 values of both groups were increased, microbial invasion of the amniotic cavity was associated with a significant increase in the amniotic fluid MMP-7 concentration between the two groups.<sup>19,20</sup>

The results of this study did not find any significant differences in the features of qualitative serum CRP and serum MMP-7 between preterm and term pregnancies with premature rupture of membranes at the Regional General Hospital Dr. Zainoel Abidin Banda Aceh. Nonetheless, CRP and MMP-7 examinations can be used as markers of potential predisposing factors for PROM and PPRM cases.

Referring to the analysis of the relationship between plasma MMP-7 levels and qualitative CRP in PROM patients with term pregnancy, based on the Mann-Whitney U test, it was found that there was a significant relationship between plasma MMP-7 and qualitative CRP in PROM patients ( $p < 0.05$ ). However, statistically based on the Independent sample t-test, it was found that there was no significant relationship between plasma MMP-7 and qualitative CRP in PPRM patients ( $p > 0.05$ ). From these two analyzes, in either PROM or PPRM, it is clear that plasma MMP-7 levels were more elevated in the CRP-negative group although in the CRP-positive group there was also an increase in MMP-7.

Physiologically, there is an increase in the concentration of MMP-7 with increasing

gestational age. However, this is precisely the opposite of the results of this study in PROM conditions, MMP-7 levels were higher in preterm pregnancy (much younger gestational age) than in term pregnancy (6.03 vs 4.6 mg / L). This interpretation is consistent with the observation that microbial invasion of the amniotic cavity at term is not associated with a significant increase in MMP-7 nor CRP. Proinflammatory cytokine concentrations were significantly lower among patients with microbial invasion of the amniotic cavity in term pregnancy than in preterm patients with premature rupture of membranes.<sup>19</sup>

### CONCLUSION

In this study, there was no significant difference in the qualitative serum CRP and MMP-7 values between preterm and term pregnancies with premature rupture of membranes. In terms of the relationship between CRP and MMP-7, it was found that there was a significant relationship between serum qualitative CRP and MMP-7 in patients with PPRM. However, it did not have a significant relationship in patients with premature rupture of membranes with term pregnancy.

### SUGGESTIONS

It is hoped that there will be further studies with a larger number of research subjects as well as further multivariate studies to evaluate how strong the correlation between CRP, MMP-7, and other parameters related to examining the presence of microbial invasion as a marker of infection has occurred. It is hoped that CRP examination can be performed as a standard examination during antenatal care to predict the occurrence of PROM in patients with a high risk of infection.

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### CONFLICT of INTERESTS

None to declare

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