C-Reactive Protein Concentration in Very Early, Early and Late Preterm Labour

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Abstract

Objective: Preterm labor (PTL) is related to neonatal morbidity and mortality. The etiology of PTL is multifactorial, however maternal inflammation is suspected to play a large role. Research has indicated a relationship between the increase of C-reactive protein (CRP), a biomarker of general tissue inflammation to the incidence of preterm labor. This study aimed at examining the relationship between preterm labor and CRP levels.

Method: This was a case-control retrospective study. Cases were patients presenting with preterm labor who came to the Department of Obstetrics and Gynecology of Hasan Sadikin Hospital Bandung. Patients were classified into very early preterm, early preterm, late preterm; control group was taken from patients without delivery complication (n=20/group). CRP serum was examined using immunoassay method.

Result: CRP median value in the early preterm group was greater than very early preterm, early preterm, and control (8.15 mg/L vs 6.5 mg/L vs 5.6 mg/L vs 5.75 mg/L, respectively) but statistical significance was not achieved (p> 0.05). Further comparisons between the very early, early preterm vs control and late preterm vs control groups were performed and no statistical significance was found.

Conclusion: Further research is required to investigate the link between maternal CRP and preterm labor.

Key words: C-reactive protein, preterm labor

Konsentrat Protein C-Reaktif (PCR) pada Persalinan Prematur Sangat Awal, Awal, dan Terlambat

Abstrak

Tujuan: Persalinan prematur memiliki kaitan yang erat dengan morbiditas dan mortalitas neonatus. Etiologi persalinan prematur ini dipengaruhi oleh multifaktor. Namun, inflamasi maternal menjadi salah satu faktor yang dicurigai paling mempengaruhi. Beberapa penelitian melihat adanya hubungan antara peningkatan Protein C-Reaktif (PCR), biomarker untuk inflamasi jaringan secara umum, dengan insidensi persalinan prematur. Penelitian ini bertujuan untuk melihat relasi antara kadar PCR dengan kejadian persalinan prematur.

Metode: Penelitian ini menggunakan metode kasus kontrol (case control). Kasus berasal dari pasien dengan persalinan prematur yang datang ke Departemen Obstetri dan Ginekologi RS Dr. Hasan Sadikin Bandung. Pasien dikelompokkan menjadi 3 kategori, yaitu persalinan prematur sangat awal, awal, dan terlambat. Kelompok kontrol diambil dari pasien yang menjalani persalinan tanpa komplikasi (n=20/kelompok). Serum PCR dianalisa menggunakan metode uji imunoserologi (immunoassay).

Hasil: Nilai median PCR pada kelompok prematur awal lebih besar daripada kelompok prematur sangat awal, awal, dan kontrol (secara berurutan, 8.15 mg/L vs 6.5 mg/L vs 5.6 mg/L vs 5.75 mg/L), namun tidak signifikan secara statistik (p>0,05). Perbandingan lebih lanjut antara prematur sangat awal, awal, dengan kelompok kontrol serta prematur terlambat dengan kelompok kontrol dilakukan dan tidak signifikan secara statistik.

Kesimpulan: Penelitian lebih lanjut dibutuhkan untuk melihat hubungan antara kadar PCR maternal dengan persalinan prematur.

Kata kunci: protein C-reaktif, persalinan prematur
Introduction

Preterm Labor (PTL) is the highest contributor to neonatal mortality and morbidity. There are a number of risk factors of PTL, including inflammation and maternal infection, maternal age, parity, multiple pregnancy, and uterine abnormalities. Even though there are various etiologies of preterm labor, intrauterine infection is estimated to contribute 40% of all incidences of PTL.

C-reactive protein (CRP) is a biomarker synthesized in the liver under inflammation and tissue injury in the body. Research in the past two decades have indicated that there is a relation between the concentration of CRP and the incidence of preterm labor, but the current data is still unable to confirm the value of CRP as a biomarker which can predict the incidence of preterm labor. Hvislom (2002) found significant differences of CRP median concentration between mothers who gave birth before term in comparison to the normal labor, but the range of CRP levels in PTL cases and in normal labor overlapped. This data is supported by Pitiphat et al. (2005), who found that the increasing levels of CRP at early gestational age was related to an increase in Preterm Labor outcomes. Nevertheless, further research by Bullen et al. (2013) in the Pregnancy Outcomes and Community Health (POUCH) trial indicated that the association of CRP and spontaneous preterm birth outcome can be confounded by maternal obesity; which was thought to occur due to a chronic proinflammatory condition mediated by adipokin. The CRP value as an independent PTL predictor is still subject to investigation, considering the result of the study which combines another inflammatory cytokine examination like IL-6 and TNF-α obtained a better predictive result than CRP independently.

Park’s retrospective study data (2018) compared the performance of IL-6 in amnion liquid compared to CRP serum in women diagnosed with choriomnionitis found that the levels of IL-6 independently functioned similarly to predict PTL outcomes compared to CRP serum. Therefore, there is a need for further research on the performance of maternal CRP serum levels in women with preterm labor.

Method

Research population

Samples were taken from was all preterm patients, defined as 28 weeks to 36 weeks from the first day of the last menstruation at the menstruation cycle of 28 days and term delivery who attended the Obstetrics and Gynaecology Department of Hasan Sadikin Hospital Bandung. Case inclusion criteria are gestational age > 28 weeks - 36 weeks, counted from the first day of the last menstruation. Patients with diabetes mellitus, cardiovascular disease, cervical incompetence, history of premature delivery, hypertension, neuropathy, and impaired renal function were excluded.

This study had been approved by the Health Research Ethics Committee, Faculty of Medicine, Padjadjaran University, Dr. Hasan Sadikin Hospital Bandung. All participants were asked for verbal and written consent after the goals and methods of the research were explained.

Research sample selection

Observational analytic case-control design was applied in this study. All case and control samples were selected based on inclusion and exclusion criteria. The samples were taken using consecutive sampling method. Power calculation was performed, and the number of 20 patients per group was reached, so that the total number of samples was 80 for the very early, early, late preterm and control groups. Classification of preterm labor was based on
the World Health Organization classification: very early preterm is defined as labor <28 weeks; early preterm 28 to <32 weeks; and late preterm 32 - 37 weeks.9

Assessment of C-Reactive Protein

Blood samples were taken from each subject through venous puncture 3 ml of blood were taken and placed in tubes. After 30 minutes, the tubes were centrifuged at 1673 g for 15 minutes and separated into 2 aliquots of 0.5-1 mL each. The aliquots were stored at the temperature of – 200°C for Electrochemiluminescence Immunoassay (ECLIA) tests.

Statistical analysis

Data normality was examined using Kolmogorov-Smirnov test. Categorical data were compared using Chi-square test, whereas continuous data were compared using Kruskal Wallis test. Pairwise comparison between case groups were conducted using Mann-Whitney test. P value <0.05 is considered to be significant.

Result

The characteristics of the subjects are displayed in Table 1. Most of the samples were between 21-34 years old (68.8%); 10% were <20 years old, and 21.3% were >35 years old, and there were no significant differences in the proportion of maternal age among all groups (p > 0.05, Chi-square test).

| Characteristics | Value | p-value |
|-----------------|-------|---------|
| Maternal Age    |       |         |
| <20 years       | 8     | 0.679*  |
| 21-34 years     | 55    | (68.8%) |
| >35 years       | 17    | (21.3%) |
| Total           | 80    |         |
| Parity          |       | 0.1**   |
| 0               | 41    | (51.2%) |
| 1               | 24    | (30%)   |
| 2               | 13    | (16.3%) |
| 3               | 2     | (2.5%)  |
| Total           | 80    |         |

*Chi-square test **Kruskal-Wallis test

Data normalization using the Kolmogorov-Smirnov test indicated that the data distribution was not normal, and thus continuous data were analysed using the non-parametric Mann-Whitney U test to compare the preterm and term groups, which was followed by the Kruskal-Wallis test to compare the very early preterm, early preterm, late preterm, and control. Next, pairwise analysis among all groups were performed using the Mann-Whitney test. The results are presented in Table 2.

There were no significant differences in the age of patients among all groups (p>0.05) (Table 1). The median of CRP levels in the preterm group was higher than
### Table 3 Comparison of Serum CRP among Very Early, Early, and Late Preterm

| Characteristics | Categories | Very Early Preterm (n =20) | Early Preterm (n =20) | Late Preterm (n=20) | Control (n =20) | p-value** |
|-----------------|------------|---------------------------|----------------------|---------------------|----------------|---------|
| Age |  |  |  |  |  | 0.679 |
| <20 years |  | 1 | 2 | 2 | 3 |  |
| 21-34 years |  | 15 | 16 | 12 | 12 |  |
| >35 years |  | 14 | 2 | 6 | 5 |  |
| Parity |  |  |  |  |  | 0.1 |
| 0 |  | 8 | 7 | 16 | 10 |  |
| 1 |  | 9 | 10 | 1 | 4 |  |
| 2 |  | 3 | 3 | 3 | 4 |  |
| 3 |  | 0 | 0 | 0 | 2 |  |
| CRP (mg/L) |  | Mean±SD | 15.83±23.36 | 10.25±7.69 | 12.81±26.13 | 8.25±7.38 | 0.695 |
| Median |  | 6.5 | 8.15 | 5.6 | 5.75 |  |
| Min |  | 1.2 | 1.6 | 1.61 | 1.4 |  |
| Max |  | 91.6 | 31.1 | 121 | 29.5 |  |
| Interquartile range |  | 9.7 | 10.45 | 7.85 | 9.7 |  |

#### Pairwise Comparison

| p-value* | Very Early Preterm | Early Preterm | Late Preterm | Control |
|----------|--------------------|---------------|--------------|---------|
| Very Early Preterm | 0.76 | 0.49 | 0.56 |  |
| Early Preterm | 0.76 | 0.26 | 0.47 |  |
| Late Preterm | 0.49 | 0.26 | 0.67 |  |

Results are based on Chi-square test for categorical data; Mann-Whitney U and Kruskal-Wallis for continuous data.

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**Figure 1** CRP Concentration among Groups
the term group (7.3 mg/L vs 5.75 mg/L, respectively) but Mann-Whitney U test did not find any significant differences (p>0.05) (Table 2). The median of CRP levels in the early preterm group was higher than those in the very early preterm, late preterm, and control groups (8.15 mg/L vs 6.5 mg/L vs 5.6 mg/L vs 5.75 mg/L, respectively) (Table 3) but the Kruskal-Wallis test did not detect a significant difference (p<0.05) (Table 3, Figure 1). Further pairwise comparison analysis between groups did not yield statistical significance (Table 3) (p>0.05).

Discussion

The lack of maternal serum CRP baseline data during each stage of pregnancy in the normal population made it difficult to determine the optimal time to assess CRP levels that are predictive to preterm labor outcomes. Pregnancy itself can be pro- and anti-inflammatory depending on the stage of pregnancy, and previous studies have shown that CRP levels in maternal serum are higher in normal pregnant women than in non-pregnant women and increased during normal labor. Timing of blood collection may also be confounded the transient nature of serum CRP levels, as with its short half-life, as Hviplot (2002) had highlighted previously; it makes it difficult to identify the time when serum CRP levels begin to increase and the optimal timepoint along pregnancy to predict PTL outcome.3

Several studies conducted prior to this study have tried to test CRP levels on preterm labor outcomes at the beginning and middle of pregnancy, with the finding of an association between the concentration of CRP in maternal serum in early pregnancy with labor before 37 weeks. This might explain the differences in the results of our study, which did not find any significant difference between all case groups and controls.

The result of this study may also be affected by our relatively smaller number of samples compared to other studies (n = 20 per group), as well as the presence of several outlier data points well above the median of groups that skew the data distribution. This can be seen from the 75th percentile of our data (13.25 mg/L) which is quite high compared to the data obtained by Pitiphat (4.85 mg/L) and the maximum value of CRP levels in our sample (121 mg/L) (Figure 1) which was not found by Hviplot, Pitiphat, and Lohsontoorn. However, it is not known whether the variability of the results of this study is due to differences in demographics and the time of blood collection.

Ghezzi et al. (2002) had assessed the relationship between maternal CRP serum levels with early (<34 weeks) and late preterm (> 34 and <37 weeks) outcomes. Our results agreed with their findings, although they had assessed their CRP levels along 15-18 weeks. However, while they too concluded that early intrauterine inflammation plays a role in preterm labor based on the increased level of amniotic fluid CRP, their analysis of maternal serum CRP and spontaneous preterm labor was based on a small fraction of their samples (< 37 weeks n = 26, < 34 weeks n = 10).

The second risk factor for preterm labor is maternal age. In our study, the age of the patients in the preterm and term birth women was quite homogeneous, therefore excluding the possibility of confounding due to the age of the sample. The strength of our study is the homogeneity between case and control samples. Yet, the relatively smaller number of samples makes further analysis more difficult to do. Further research needs to be done involving larger samples and taken at different points of time to signify the link between CRP and preterm labor.

Conclusion

This study has not been able to conclude
that there is a relationship between CRP and preterm labor outcomes. There is still a need to conduct further research with larger samples.

Declarations
Ethical Approval and Consent to Participate

This study protocol was approved by Faculty of Medicine, Universitas Padjadjaran, Ethics Committee Review Board and all study participants gave verbal and written informed consent. All authors hereby declare that all patients have been examined in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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