

C-reactive protein as a biochemical marker for idiopathic preterm delivery

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Abstract

Background and objective: Estimation of C-reactive protein is a simple method and could be helpful in detecting cases at risk for pre-term delivery. In this study we examined the association of maternal C-reactive protein in second trimester with risk of occurrence of preterm delivery later on.

Methods: A prospective case-cohort study was conducted between June 2011 and July 2012 at Maternity Teaching Hospital Iraq- Erbil. The sample size was 205 women at gestational age of 20⁺⁰ weeks to 26⁺⁶ weeks and after exclusion of all risk factors for preterm delivery. High-sensitivity C-reactive protein assays were performed on serum samples.

Results: Median concentration of C-reactive protein was 4.7 mg/liter. No significant association was found between high level of C-reactive protein and occurrence of preterm delivery ($P = 0.78$).

Conclusion: Measurement of C-reactive protein in second trimester cannot be used as an indicator for pre-term delivery.

Keywords: C-reactive protein, premature uterine contractions, premature delivery, pre-term delivery.

Introduction

Preterm births occur in approximately 12% of pregnancies and it is the leading cause of neonatal mortality in the United States.^{1,2} In addition to the high perinatal mortality, the risk of later neurodevelopmental impairment, disability and handicap are especially significant within the 24-26 weeks gestational age window.³ Up to 40% of survivors born before 26 weeks gestation were found to have a head circumference below 3rd centile at two years of age after which little catch up growth is possible.⁴ Successful reduction of perinatal mortality and morbidity associated with prematurity may require implementation of programs for accurate identification of pregnant women at risk. In fact, recent evidence suggests that early identification of at-risk pregnancies with timely referral for sub specialized obstetrical care may decrease morbidities of extreme prematurity (<32 weeks).⁵

A short or partially dilated cervix may allow bacteria to ascend into the lower pole of the uterus where inflammatory response will be triggered. This then leads to cervical ripening and shortening, decreasing the ability of the cervix to act as a mechanical or microbiological barrier and, ultimately leads to the development of either localized or generalized chorio-amnionitis and leads to preterm delivery.⁶ However, it's also possible that with a high number of virulent pathogens in the vagina, bacteria may gain access to the lower pole of the uterus through a normally functioning cervix and then ripening of cervix will happen. Bacteria may also gain access to the amniotic cavity through hematogenous spread or by introduction at the time of invasive procedures.⁶ Hematogenous spread can also be from oral bacteria although studies of intensive dental hygiene in pregnancy have not shown to reduce the risk.^{7,8} The earliest births carry

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the highest risk of an infection as etiology.⁹ Although the patho-physiology of preterm delivery remains unknown, importantly, some investigators have indicated that infections are major causes of preterm deliveries (PTD), responsible for about 30%-50% of all cases.^{10,11} Moreover there is increasing evidence, which suggests that other infectious processes occurring elsewhere in the body may contribute to preterm delivery.^{12,13} C-reactive protein (CRP) is a protein found in the blood, the levels of which rise in response to inflammation.¹⁴ It is an acute-phase reactant protein synthesized primarily by liver cells in response to the pro-inflammatory cytokines including interleukin-6 and TNF- α .^{15,16} CRP concentrations in peripheral circulation are also known to be associated with high body mass index (BMI) and other markers of adiposity.^{17,18} On the same basis ladies with BMI ≥ 30 were excluded from the study. Elevation of C-reactive protein in maternal peripheral circulation is associated with the presence of intrauterine infection.^{19,20} Endothelial dysfunction has been postulated to be part of an exaggerated maternal inflammatory response to pregnancy.²¹ In addition, cross-sectional analyses indicate that CRP is strongly correlated with markers of endothelial activation and dysfunction.²² Collectively, these findings, suggest that C-reactive protein, a marker of systemic inflammation, may be involved in the pathogenesis of preterm delivery. Hvilsom et al in 2002²³ were among the first group of investigators to report that elevated concentrations of CRP in maternal serum during early pregnancy (≥ 85 th percentile vs. <85 th percentile) were associated with a two-fold increased risk of preterm delivery (OR = 2.0, 95% CI: 1.2-3.5). Some others have corroborated this early finding,^{24,25} though not all investigators.²⁶ The aim of this study was to assess the association of CRP, a marker of inflammation, with preterm delivery, to examine the reliability of CRP in maternal serum as a marker of premature birth among pregnant

women who had no of the known risks for premature birth and to determine critical value of CRP in pregnancies that ended up with a premature birth.

Methods

The population for the present analysis was drawn from the attendants of antenatal care clinic at Maternity Teaching Hospital in Erbil/Iraq in the period from June 2011 to July 2012. Women eligible for inclusion were those who started antenatal care prior to 20 weeks gestation, were ≥ 18 years old, BMI <30 , could speak Kurdish or Arabic, at gestational age between 20⁺⁰ and 26⁺⁶, planned to carry the pregnancy to term, and to deliver at study hospital. Both nulliparous and multiparous women were approached and enrolled. They were asked to take part in an hour-long interview in which trained personnel used a structured questionnaire to get information about maternal socio-demographic characters, maternal age, occupation and reproductive and medical history. Participants also provided non-fasting blood sample for estimation of CRP, high vaginal swab, mid stream urine for analysis and culture and sensitivity, and trans-vaginal sonography for measurement of cervical length. The BMI was taken from booking records. Pregnancy outcome information was ascertained by a direct contact with the participants. During the study period, 208 eligible women were approached, and all gave a well-informed verbal consent. Three women were lost from follow up and were excluded from the analysis. Any woman at risk for pre-term birth such as having a history of pre-term birth, cervical incompetence, urinary tract infection (proved by urine test), bacterial vaginosis, diabetes (gestational or non gestational), hypertension, multiple gestations, poly-hydramnios, uterine congenial abnormality, placenta previa, and BMI ≥ 30 was excluded from the study. Gestational age was based on the last menstrual period (LMP) and confirmed by ultrasound examination,

conducted prior to 20 weeks gestation. If both last menstrual period and ultrasound dating were available and the difference between ultrasound and LMP exceeded one week then the ultrasound dating was used to assign gestational age. We categorized preterm delivery cases according to gestational age. Serum CRP levels were measured by quantitative highly sensitive immunoassay test (the i-CHROMATM CRP Test along with i-CHROMATM Reader). It is a fluorescence immunoassay that measures CRP in serum, plasma, and whole blood. A single technician and a single laboratory specialist did all the tests by ELISA method. The lab staff members were blinded for the blood samples. Maternal non-fasting blood samples were collected at gestational age 21.8 (The mean gestational age at time of sampling) in 10 ml test tube without anticoagulant and allowed to clot. The serum was then removed from the sample as soon as possible to avoid hemolysis. Samples were kept frozen until tested. A levels ≥ 10 mg/l was considered being high. Patients were followed up until delivery and the gestational age at the time of delivery was noted.

Statistical analysis and ethical consideration: The distribution of maternal socio-demographic characteristics, medical and reproductive histories according to preterm and term delivery status was examined. To estimate the relative association between varying concentrations of CRP and risk of preterm delivery, the data

was analyzed using the statistical package for the social sciences (version 18). Student's t test was used to compare between means and Chi square test of association was used to compare between proportions. *P* value of ≤ 0.05 was considered statistically significant. The scientific and ethical committee of the Council of Arab Council for Medical Specialties approved the study.

Results

Approximately 11.7% of the women (24 of 205) in this study delivered preterm. Among preterm deliveries, 95.8% were preceded by spontaneous premature uterine contractions. Approximately 4.17% were classified as medically indicated preterm delivery with no records for pre-labor preterm rupture of membrane (PPROM) as shown in Table 1. Table 2 represents the results according to the CRP values in which < 10 is normal and ≥ 10 is abnormal depending on the laboratory standard ranges. We found that 167 of the cases had normal CRP values (< 10); 19 (11.4%) ended preterm and 148 (88.6%) delivered at term. Of the 38 cases with abnormal value (≥ 10), 5 (13.2%) pregnancies ended preterm and 33 (86.8%) delivered at term. The total preterm deliveries were 24 (11.7%) and the total term deliveries were 181 (88.3%). There was no statistically significant relation between high CRP value in second trimester of pregnancy and preterm delivery ($P = 0.781$).

Table 1: The total preterm deliveries and the pathophysiological classification.

Total pre-term deliveries	Spontaneous Pre-term deliveries		Medically indicated pre-term deliveries		PPROM	
Total no.	No.	%	No.	%	No.	%
24	23	95.83	1	4.17	0	0

Table 2: CRP level in relation with pre-term delivery.

CRP mg/l	Delivery				Total		P value
	Pre-term		Term		No.	%	
	No.	%	No.	%			
< 10	19	11.4	148	88.6	167		0.781
≥ 10	5	13.2	33	86.8	38		
Total	24	11.7	181	88.3	205		

Table 3 shows the relation between the CRP values and the different variables in the study, which shows no significant relation. Both primi-gravida and multi-gravid was were included in the study and there was no significant relation between parity and CRP level as shown in Table 4. Table 5 shows the relation between very high level of CRP and preterm labor. In this

table we classified our results into three CRP values. We found that preterm deliveries were 19 (11.4%) when CRP was <10, and became 2 (10%) when CRP was between 10 and 19 and then 3 (16.7%) when CRP was ≥ 20 . The percentage of preterm delivery was highest in very high CRP value (i.e. ≥ 20) but this was not statistically significant.

Table 3: The relation between CRP and variables.

CRP level mg/l	≥ 10 (N = 38)		<10 (N = 167)		P value
	Mean	SD	Mean	SD	
Age in years	28.37	5.70	27.81	6.46	0.63
Cervical length in mm	33.62	7.10	33.97	5.30	0.73
Number of previous abortions	0.18	0.61	0.29	0.61	0.32

Table 4: The relation between parity and CRP value.

CRP mg/l	≥ 10 (N = 38)		<10 (N = 167)		Total (N = 205)
Primigravida	16	42.1%	63	37.7%	79
Multigravida	22	57.9%	104	62.3%	126
P value	0.721				

Table 5: Pregnancy outcome in relation with the level of CRP.

CRP mg/l	Pregnancy outcome				Total	P value
	Term		Pre-term			
	No.	%	No.	%	No.	%
<10	148	88.6	19	11.4	167	100
10-19	18	90	2	10	20	100
≥ 20	15	83.3	3	16.7	18	100
Total	181	88.3	24	11.7	205	100

Discussion

In the current study we found that there was no association between mid-pregnancy maternal serum CRP and preterm delivery as the mean and the median of CRP in preterm deliveries (8.09 and 6.59 mg/l, respectively) did not differ significantly from the mean and median serum CRP in term deliveries (7.05 and 4.64 mg/l, respectively). This finding was similar to Ghezzi F et al²⁷ in which no relationship was found between maternal blood C-reactive protein concentration and preterm delivery. Another study done by Borna et al²⁸ showed no correlation between amniotic fluid high sensitive-CRP concentrations with preterm delivery. Ozer et al²⁹ did not find any difference with respect to mean high sensitive-CRP levels between term and preterm deliveries and high sensitive-CRP levels higher than 5 mg/l (75th percentile) had sensitivity of 30%, specificity of 87% in the prediction of spontaneous preterm delivery at <37 weeks. While Pitiphat et al²⁴ enrolled 2,670 consenting women between April 22nd, 1999, and July 31st, 2002, of whom 329 subsequently became ineligible because of multiple gestation (n = 19), because obstetric care was transferred to a non study site (n = 115), or because the women were no longer pregnant (n = 195). Of the 2,341 remaining participants, 195 (8%) withdrew and 18 (<1 percent) were lost to follow-up. For this analysis, of the 2,128 participants who delivered a live infant, they included the 2,088 women who also provided blood samples in early pregnancy. They found no significant association between quartiles of CRP and preterm delivery. They also found that very high levels of maternal plasma CRP in early pregnancy were associated with increased risk of preterm delivery. In this study we found that the percentage of preterm delivery in very high level of CRP ≥ 20 mg/l (21.4%) was more than that in CRP level of <20mg/l (11%) but this finding was not statistically significant. This may be due to small sample size, as only five pregnant

ladies who delivered pre-term had high CRP value, and only three cases had very high CRP level. Hvilsom et al,²³ in their prospective case-control study of cohort of 84 singleton preterm cases and 400 term controls, the authors reported that women with CRP concentrations $\geq 85^{\text{th}}$ percentile (i.e., ≥ 7.6 mg/l) experienced a two-fold increased risk of preterm delivery (OR = 2.0, 95% CI: 1.2 - 3.5) compared with women who had lower CRP concentrations. However, the authors did not assess the risk of different sub-types of preterm delivery in relation to varying concentrations of CRP. The etiology of preterm delivery is undoubtedly multi-factorial. It is this multi-factorial etiology that raised difficulties and differences in determining the effectiveness of various markers in the prediction of preterm delivery.^{30,31} Differences in study design, timing of blood collection, the underlying characteristics of the study population, and incomplete or no control for confounding may account for the variability in results across studies. For instance, investigators have noted that serum CRP concentrations measured in the third trimester (28 weeks gestation) is more strongly associated with preterm delivery, while concentrations measured in serum collected upon entry to prenatal care (first and early second trimesters) appear to be less strongly associated with preterm delivery, therefore we designed this study to measure CRP in the middle and late second trimester so we can add some data to those previous studies. The results of this study implicate serum-CRP in the mid second trimester, was not a possible risk marker of preterm delivery. This study had some limitations. First, a single measurement of serum CRP is not likely to provide a time-integrated measure of maternal inflammation status during the whole pregnancy. Second, the relatively small sample size of the study. Thus the relatively small number of cases who delivered preterm, especially those with very high CRP values, affected the results.

Conclusion

Very high CRP levels exceeding the threshold ($\geq 20\text{mg/l}$) might be associated with increased risk of preterm delivery independent of many other determinants of pre-term delivery. The association was apparent primarily for spontaneous preterm delivery but not significant. Future studies should evaluate whether changes in CRP levels over the course of pregnancy are predictive of at risk pregnancies for pre-term labor and to find the best time of pregnancy to do CRP screening in order to sort out the pregnancies at risk.

Conflicts of interest

The authors report no conflicts of interest.

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