Level of C - reactive protein as an indicator for prognosis of premature uterine contractions

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Summary

Background and objectives: **high concentrations of maternal C-reactive protein have been associated with adverse pregnancy outcome, and premature uterine contraction may be predicted by elevated levels of C-reactive protein. This may ultimately be simple and cost-effective enough to introduce as a low-risk screening program.**
Patients and methods: **an observational case control study was performed from May 1st, 2010 to December 1st, 2010 at Maternity Teaching Hospital-Erbil/ Kurdistan Region/ Iraq. The sample size was (200) cases. Hundred of them were presented with premature uterine contractions at 24^{+0}-36^{+6} weeks. The other hundred were control group at same gestational ages. The level of C-reactive protein was determined in both groups and both groups were followed till delivery.**
Results: **(93) out of (100) women with premature uterine contractions had elevated level of C-Reactive protein and 91% delivered prematurely while in the control group only (9) out of (100) women had elevated level of C-reactive protein and only 8% of them delivered preterm. Differences were statistically highly significant.**
Conclusion: **C-reactive protein can be used as a biomarker in prediction of premature delivery when it is associated with premature uterine contractions. As well it can be used as a screening test to detect cases that are at risk of premature delivery.**

Key words: C-reactive protein, premature uterine contractions, premature delivery, preterm delivery.

Introduction

Preterm uterine contractions defined as regular uterine contractions at less than 8 minutes interval, but which do not fulfill the criteria for preterm labor (1). These criteria are documented by American Academy of Pediatrics and the American College of Obstetrics and Gynecology which include the followings: Regular uterine contractions occurring at a frequency of four in 20 minutes or eight in 60 minutes (or at interval of < 8 minutes) plus progressive changes in the cervix; cervical dilatation greater than 1 cm; and cervical effacement of 80% or greater (2). Diagnosis of preterm labor is one of the most difficult and important tasks facing clinicians' today (1). The cardinal symptom of impending delivery is uterine contractions pain, which typically increase in frequency and intensity as labor progress. Too few studies have evaluated factors associated with maternal perception of uterine contractions (3), as perception of uterine contractions allows women to reach medical evaluation and intervention.
Complications of preterm uterine contraction include unattended births, prematurity, hemorrhage, infection, impaired childhood development, and peri-natal death (4).
CRP is a sensitive marker of systemic inflammation (5-7). CRP accompanies both acute and chronic inflammatory disorders (8). Serum concentrations of CRP in pregnancy are elevated above non pregnant values, with the difference being detected as early as 4 weeks gestation (9). The exact etiology of this increase is unknown, although the direct synthesis of CRP by trophoplast may play a role (10). Higher concentrations of CRP in the 1st trimester have been associated with preterm delivery (8). Maternal concentrations of CRP have been studied as an aid in diagnosing subclinical infection in pregnant women who experience preterm labor and premature rupture of membranes. In the past decade elevated levels of CRP measured during gestation have been linked to adverse pregnancy outcomes such as preeclampsia and intrauterine growth restriction and have been associated with the presence of intrauterine infection (2). Systemic maternal infections can lead to cervical ripening and premature delivery through inflammatory cytokines then prostaglandin production (11).

In UK preterm birth divided into three gestational periods, mildly preterm births at 32^{+0} to 36^{+6} wks (incidence 5.5%), moderately preterm births at 28^{+0} to 31^{+6} weeks (incidence 0.7%) and extremely preterm births at 24^{+0}-27^{+6} weeks (incidence 0.4%).Incidence of preterm birth of all UK births is 6.6 - 12%. Significant high rates of preterm birth of 11% are reported from USA. In many Nordic countries the incidence is below 5% (12).

Preterm birth can be caused by infection. Systemic maternal infections have been associated with preterm delivery. There are findings that suggest that very high CRP levels in early pregnancy are associated with preterm delivery. Inflammation without infection may cause preterm birth on its own, as intra amniotic infusion of interleukin-1B cause's uterine contractility in primate (13). Bacterial vaginosis is a possible cause of preterm birth characterized by an altered vaginal microbial flora (14). Numerous studies report an incidence of 15-20% among pregnant women and emphasized the importance of this infection in development of preterm delivery, premature preterm rupture of membranes (PPROM) and post partum endometritis (15, 16).

Objectives

To identify association between the level of CRP and preterm delivery, whether CRP can be used as a biomarker to predict premature delivery when the patient has premature uterine contractions in order to improve prognosis of premature uterine contractions and detection of cases of premature labor at early stage when it still can be prevented and whether measuring serum CRP can be used as a screen test to detect pregnant women who are at risk of preterm delivery.

Patients and methods

This is a prospective case control study. The study was conducted at Maternity Teaching Hospital in Erbil City/Kurdistan region/Iraq, from May 1st til December 1st of 2010. Sample size was 200 cases divided into two groups. Group 1 was a hundred (n_1=100) healthy pregnant women with singleton pregnancy, presented with premature uterine contractions at gestational age between 24^{+0}-36^{+6} weeks. The other hundred (n_2=100) were control group (group 2) at same gestational ages. Premature uterine contractions were confirmed clinically and by cardiotocography for gestation more than 32 weeks. All women had intact membrane based on history, speculum examination and ultrasound examination to measure amniotic fluid index. Both groups were subjected to quantitative high sensitivity CRP test. Patients with concurrent conditions that may cause elevated serum CRP level as gestational diabetes, diabetes mellitus, essential hypertension, pre-eclampsia, antepartum hemorrhage and systemic chronic illness and infections (respiratory, renal and cardiovascular) and medically induced premature uterine contraction (iatrogenic) were excluded.

This study was approved by the scientific committee at College of Medicine/ Hawler medical university and an informed verbal consent was obtained from the participants. The data were gathered through validated questionnaire based on available data in libraries, habits and accessible articles. The questionnaire was filled out through direct interview. General physical and obstetrical examination was done for every patient. Vaginal speculum examination, abdominal ultrasonography for measurement of AFI, trans-vaginal ultra-sonography for measurement of cervical length, high vaginal swab for evidence of bacterial vaginosis, CRP test, general urine examinations and culture and sensitivity were performed. The gestational age was

Table 1. Demographic and obstetric characteristics of the participant.

Variables		Group 2 (n=100)	Group 1 (n=100)
		No. and Percentage (%)	No. and Percentage (%)
Age (years)	18-24 years	24(24%)	31(31%)
	25-30 years	23(23%)	28(28%)
	>=30 years	53(53%)	42(42%)
Parity	primigravidae	25(25%)	28(28%)
	Multigravidae	75(75%)	72(72%)
Occupation	Housewife	74(74%)	90(90%)
	Manual worker	1(1%)	10(10%)
	Office worker	25(25%)	0
Gestational age (weeks)	24^{+0}-27^{+6}	19(19%)	7(7%)
	28^{+0}-31^{+6}	42(42%)	37(42%)
	32^{+0}-36^{+6}	39(39%)	56(56%)
Cervical length (cm)	< 2.5	11(11%)	23(23%)
	>= 2.5	89(89%)	77(77%)

calculated from the date of the LMP and confirmed by early second trimester ultrasonographic examination before 20 wks gestation.

Laboratory methods of measuring CRP

The level of CRP measured through a quantitative highly sensitive immunoassay test (The i-CHROMA™ CRP Test along with i-CHROMA™ Reader is a fluorescence immunoassay that measures CRP in serum, plasma, and whole blood). Measurement was done by the same technician and the same laboratory specialist by ELISA method. The laboratory personnel were blinded for the blood samples. Blood samples were collected from the pregnant women in a test tube without anticoagulant and allowed to be clotted. The serum removed from the clot as soon as possible to avoid hemolysis then the samples were kept frozen until tested by the lab. Levels more than 1 mg/l was considered to be high, as this was the laboratory's standard and CRP value of <1 is abnormal according to that specific laboratory therefore this was used as the cut off value to test the utility of C-reactive protein as predictor marker of premature delivery in patients with premature uterine contractions. Patients were followed up until delivery and the gestational age at the time of delivery was noted.

Data analysis: data were analyzed by Predictive Analytical Software (Microsoft Statistical Package for Social Sciences; SPSS for windows 18.0 version). P value < 0.05 was considered to be significant. Chi-square & Students t test were used for analysis.

Results

The mean age in women with premature uterine contractions was 27.70±5.86 years, while the mean age in control group was 28.95±6.10 years. Most of the participants were housewives and only one pregnant lady in the study was smoker, who had premature uterine contractions.

We observed that the mean of cervical length in cases of premature uterine contractions was 1.87 cm which is less than mean of cervical length of the control group 2.95 cm and the P <0.03.

The effect of elevated level of CRP on the outcome shown in Table 2.

It is observed that 93% of (100) pregnant women with P.U.C had elevated level of CRP.

There is a significant association between elevated level of CRP and preterm delivery when CRP elevated above 1mg /l in cases of P.U.C. than in the control group.

The mean value of CRP in cases of premature uterine contractions was 9.24±7.91 mg/l which is higher than mean value in the control group 0.92 ± 0.92 P < 0.001.

The level of CRP was significantly elevated in cases with premature uterine contractions than control group.

The following values can be obtained from this Table: Sensitivity is 98.9%, Specificity is 66.7%, Predictive value + (PV+) : 96.8 % and Predictive value –ve(PV-) is 85.7%.

Likelihood ratio of a positive test = sensitivity/1-specificity=0.989/0.333=2.96.

The following values can be obatained from this Table: Sensitivity is 50%, Specificity is 5.4%, Predictive value +: 4.4% and Predictive value - : 55.6%

Table 2. The effect of elevated level of CRP on the outcome.

CRP class	Group 2 (n=100)			Group 1 (n=100)			P value
	No. and percentage	Outcome Term	Preterm	No. and percentage	Outcome Term	Preterm	
CRP<=1 mg/l	91 (91%)	87 (95.6%)	4 (4.4%)	7 (7%)	6 (85.7%)	1 (14.3%)	**P<0.3**
CRP>1 mg/l	**9 (9%)**	**5 (55.6)**	**4 (44.4%)**	**93 (93%)**	**3 (3.2)**	**90 (96.8%)**	**P<0.001**

Table 3. Comparison of means of CRP between the two groups when CRP >1mg and when <=1mg in different age groups.

VARIABLE		Group 2 (n=100)		Group 1 (n=100)		P value
CRP group	Age group(years)	No.	Mean of CRP	No.	Mean of CRP	
CRP <=1mg/l	18- 24	22	0.68±0.20	2	0.61± 0.07	P<0.001
	25-30	22	0.60±0.28	1	0.49±00	
	>=31	47	0.68±0.22	4	0.58±0.21	
CRP > 1mg/l	18- 24	2	1.97±1.08	29	8.92±9.59	
	25-30	1	2.98±00	26	8.13±9.24	
	>=31	6	2.8±2.9	38	8.34±9.48	

Discussion

Actually there are few researches in this field therefore the results of this study may be of great value for the target population. The results show that women with premature uterine contractions who had an abnormally high level of CRP were at high risk for development of preterm labor and preterm delivery. No clinical signs of infection were found in any of the cases of pregnancy complicated by preterm delivery; however in these pregnancies the elevated level of CRP may suggest presence of a subclinical intrauterine infection.

Among hundred pregnant women included in this study presenting with premature uterine contractions; 91% of them delivered preterm within seven days of the contractions and (93%) of them had significant elevated level of CRP (level of CRP were more than 1mg/l P value < 0.001). Such high percentage may be due to elevated levels of CRP that reflect the subclinical infection which lead to production of more pro-inflammatory cytokines that are responsible for induction of labour and subsequent delivery. These results are consistent with the hypothesis that chronic low-grade inflammation may raise CRP levels and cause preterm delivery.

Pitiphat et al. (2005) (8), Lohsoonthporn et al. (2007) (17) and Czajka et al. (2004) (18) found that there is statistically significant association between CRP concentrations and subsequent preterm delivery, with odd ratios of 2.55 and 2.04, respectively in the first two studies. The elevated CRP concentrations were associated with an increased risk of delivery prior to completion of 34 weeks gestation (very preterm delivery). These findings are similar to results of the present study.

In this study we found that elevated level of CRP > 1 mg /l in pregnant women regarded as high level and women were at high risk of preterm delivery in association with premature uterine contractions while Dodd's and Iams (19) observed that a maternal CRP level of 8 mg/l or greater identified a subgroup of women at highest risk of preterm delivery.

Note that the PV+ (yield) of the test is high in Table 4 because it is done out of a population with a high prevalence of a condition, or a population with high risk. Results in Table 5 indicate that CRP measurement is not recommended as screen test for those who do not have PUC (20).

In the study of Cammu et al**.** (21) elevated CRP levels were more often found in women who were refractory to tocolysis, suggesting underlying infections morbidity. Mazor et al. (22) stated that the sensitivity and specificity of serum CRP for the detection of amniotic fluid infection in cases of preterm delivery were 71.5, 73% respectively. This study was not designed to detect intra-amniotic infection therefore an amniotic fluid was not taken for culture and sensitivity.

Regarding cervical canal length as a risk factor for preterm delivery specially when it is associated with high CRP, there are studies showing that the elevated concentrations of inflammatory markers were found to be strongly associated with the presence of short cervix less than 2.5 cm. It is possible that elevated cytokines levels initiate breakdown of connective tissue in the cervix or conversely, that a short cervix may provide easier access for ascending infection to the uterus, resulting in increase in cytokine levels as the study done in Denmark by Vogel, et al. (2006) (13) that cervical length, serum TNF- α and cervico-vaginal IL-6 are a clinically useful prediction of recurrent preterm birth in early second trimester. Another study in Philadelphia of Berghella, Iams, et al. (2004) (23) observed that in women with cervical length of < 2.5 cm at 22 -24 weeks of gestation with frequent premature uterine contractions , there was a two fold increase in the risk of preterm births. As this study was designed primarily to estimate the utility of CRP alone as indicator of preterm delivery, the relation between cervical length and CRP values was not determined in this study.

Table 4. CRP as screening test for preterm delivery among group 1.

CRP	Preterm		Term		Total		p
	No.	**%**	**No.**	**%**	**No.**	**%**	< 0.001
Positive	90	98.9	3	33.3	93	93	
Negative	1	1.1	6	66.7	7	7	
Total	91	100	9	100	100	100	

Table 5. CRP as screening test for preterm delivery among group 2.

CRP	Preterm		Term		Total		p
	No.	**%**	**No.**	**%**	**No.**	**%**	< 0.001
Positive	4	50	87	94.6	91	91	
Negative	4	50	5	5.4	9	9	
Total	8	100	92	100	100	100	

Regarding the history of urinary tract infection in the current pregnancy, we already excluded cases with sign and symptoms of infection at the time of interview; however 38 (38%) of pregnant women within the group of premature uterine contractions had positive history of UTI in current pregnancy and 35(35%) of the group delivered preterm (P <0.04) which is statistically significant in comparison with control group.
To our best knowledge previous study has evaluated cytokines as predictive markers for premature uterine contractions in the region. However the current study suggests that CRP concentrations may be a valuable predictor for high-risk women.

Limitations of the study

1. A single measurement of CRP is not likely to provide a time integrated measurement of maternal inflammation status during the index pregnancy.
2. The relatively small number of subjects available for subgroup analyses resulted in imprecise measures of associations as reflected by the very wide 95% confidence intervals.

Conclusion

- There is a strong association between the elevated level of CRP "a sensitive biomarker" and prediction of premature uterine contractions.
- Measurement of the level of C-reactive protein during pregnancy can be used as a predictive screening biomarker for detection of subclinical infections that cause preterm uterine contraction and hence early intervention and intensive antenatal care to reduce the peri-natal morbidity and mortality.

Recommendations

- Considering the importance of this subject, it is hopeful that the results of this research can be used for promoting further studies regarding CRP and its relationship with pregnancy complications and also other important factors relating CRP such as nutrition, infections, economical and social situations.
- This study can be used as infrastructure to build other studies in the field of premature uterine contractions.
- Women at risk for preterm labor should be encouraged to participate in studies aiming at early detection and treatment of premature labour.
- Future research on molecular biological techniques with high sensitivity and specificity may allow the development of multiple marker tests for the prediction of PTB in asymptomatic and symptomatic at-risk women. This may ultimately be simple and cost-effective enough to introduce as a low-risk screening program.

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