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PLASMA HIGH SENSITIVE C-REACTIVE PROTEIN IN EARLY PREGNANCY AS A MARKER OF PRETERM DELIVERY A CASE –CONTROL STUDY

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ABSTRACT

A nested case control study was carried out to evaluate the association of early pregnancy high sensitive C-reactive protein (HsCRP), a marker of inflammation, with preterm delivery. Subjects were 92 women who delivered preterm (<37 weeks' gestation) and 92 controls (term deliveries) matched on age, race/ethnicity, height and weight. High-sensitivity CRP assays which was measured by immunoturbidimetry assay and followed till delivery were performed on early-pregnancy (< 21 weeks gestation) plasma samples. Odds ratios and 95% confidence intervals were estimated by using conditional logistic regression adjusted for matching factors, gestational age at blood collection, and pre pregnancy body mass index. Median concentration of CRP was 3.65 mg/liter in cases versus 2.76 mg/liter in controls. No significant association was found between quartiles of CRP and preterm delivery. However, CRP levels >7 was significantly associated with the increased risk of preterm delivery (p <.0096;odds ratio 6.11; 95% CI1.31-28.39) These findings suggest that very high CRP levels in early pregnancy are associated with preterm delivery.

ABBREVIATIONS: CI- confidence interval; hsCRP- high sensitive C-reactive protein; OR-odds ratio.

INTRODUCTION

Although medical advances have improved the survival of preterm infants, little success has been attained in understanding and preventing preterm birth. Great efforts have been spent to characterize and define the utility of biologic fluids from various sources in predicting preterm birth. Intrauterine infections contribute a major part(40–50 percent) of all preterm births.[1] Systemic maternal infections lead to increased inflammatory cytokine levels, which in turn stimulate prostaglandin production; this process can lead to the induction of uterine contractions and cervical ripening culminating in preterm parturition. High concentrations of proinflammatory cytokines such as interleukin-6 and interleukin-8 in serum have been prospectively associated with preterm birth.[2] Measurement of circulating inflammatory markers may thus provide an alternative method of detecting women at high risk of preterm delivery. C-reactive protein (CRP) is a sensitive marker of systemic inflammation and is primarily synthesized in hepatocytes in response to infection and tissue injury.[3] Production of CRP is stimulated by the release of proinflammatory cytokines including interleukin-1, interleukin-6, and tumor necrosis factor-alpha. Maternal concentrations of CRP have been studied as an aid to diagnosing subclinical infection in pregnant women who experience preterm labor and premature rupture of membranes.[4,5] Recently, elevated levels of CRP measured during gestation have been linked to adverse pregnancy outcomes such as preeclampsia.[6,7] and intrauterine growth restriction.[9] Understanding preterm bith is challenging due to its multifactorial etiologies and pathophysiologic heterogeneity. To our knowledge, there are only few studies of the relation between CRP levels in maternal circulation during the first trimester of pregnancy and risk of preterm delivery and these available studies showed contradictory results. In light of the conflicting and scarcity of data this study was planned with an aim to establish prospectively the association between maternal plasma CRP levels in early pregnancy and risk of subsequent preterm delivery.

MATERIAL AND METHOD STUDY DESIGN

The present prospective study was carried out in the Department of Obstetrics and Gynecology in collaboration with the Department of Biochemistry Mahatma Gandhi Medical college Indore for two years. Ethical clearance was obtained from the Institutional Human Ethical committee. Patients attending the antenatal opd in their early second trimester (less than 20 weeks) were enrolled during this period. Women with history of multiple gestation, having plan to move out of the area before delivery, history of hypertension, diabetes mellitus, heart diseases, acute or chronic infections history of oral contraceptive pill, addiction like smoking, tobacco chewing or refusal to give consent were

excluded. As increased Body Mass Index (BMI) interferes with serum CRP level, we included the subjects with normal BMI between 18-24 kg/m2. Detailed present, past and obstetric histories were obtained followed by general and systemic examinations. Written informed consent was taken from patient and relative. Participants with a diagnosis of genitourinary infection at any time during pregnancy were identified according to International.

Classification of Diseases, Ninth Revision, codes from the automated medical record system; included were infections and inflammations of the kidney, urethra, urinary tract, and pelvic organs and genital organs, including sexually transmitted diseases.

Case-control selection

In this analysis, a prospective nested case control approach was used in which total 92 patients who experienced preterm delivery (<37 weeks' gestation) were taken as cases. Controls (92 pts.) were randomly selected from among those included women who delivered at 37 or more weeks' gestation, matched 1:1 to cases by age, race/ethnicity, height and weight.

Laboratory procedure

At the initial prenatal visit, each participant provided a plasma sample (5ml), that was collected in ethylenediaminetetraacetic acid, Sera were separated out by centrifuging at 3000 rpm for 10 min and stored separately at - 70C untill used for estimating C - reactive protein. by Latex turbidimetrymethod.The concentration of CRP in baseline plasma samples of cases/controls was determined with a validated high-sensitivity by Latex turbidimetry assay. A single technician, blinded to case-control status, performed all assays. This assay had a sensitivity of 0.04 mg/liter.

Statistical analysis

The data was analyzed by SPSS 13.0. Proportion test (z test) and chi - square test (x2 test) were applied for statistical analysis. Spearman correlations between CRP and selected variables was calculated. The Kruskal-Wallis test and post hoc Wilcoxon tests was used to compare median CRP levels between women who delivered before 37 weeks' gestation and women who delivered at term. Conditional logistic regression analyses was performed to evaluate the risk of preterm delivery. p value <0.05 was considered significant.

RESULTS

Incidence of preterm pregnancy was about 16.7% which was similar to other studies. Most of preterm labour occurred in primigravidas (47.8%) and second gravidas (46.7%). Majority of case were in age group 25-29 (35.9%),followed by 20-24 years(29.3%), 30-34 years(19.0%), < 20 years (10.9%) and >34 years (4.9%). Majority of patients belongs to urban area (60.9%) followed by rural area(39.1%).About 21.2% pateints had history of previous preterm pregnancy and 78.8 % had no history of previous preterm pregnancy. Maximum (36.4%) patients had CRP levels between 2-4, followed by 0-2 (31.0%), 4-6(23.4%) and 6-8(9.2%). Mean CRP levels among cases was 3.65 and mean CRP level among control was 2.76 and this differance in mean CRP level was statisticaly significant (p<0.0026)(Table 1).Total number of patients having CRP level >7 were 13. Out of 13 pateints, 11 (84.6%) delivered at preterm and only 2 (15.4%) delivered at term. This association betweenCRP level >7 and preterm delivery was statistically significant (p <.0096; odds ratio 6.11; 95% CI1.31-28.39) (Table 2) (figure 1).Thus, CRP level>7 identifies a group with higher risk of preterm delivery. Total number of patients having history of previous preterm delivery was 39. Out of 39 pateints, 24 (61.5%) delivered at preterm and only 15 (38.5%) delivered at term. This association between previous preterm delivery and risk of future preterm labour was not statistically significant (p <0.107; odds risk ratio 1.82) (table 3). Around 30.4% were LBW babies and 64.4% were of normal weight.Additional analyses comparing women at different CRP levels at or above the 75th percentile with women whoseCRP levels were below the 75th percentile (table 4) showed dose response.

Table 1: Mean CRP level among Cases and Controls

Delivery in weeks	No of patients	Mean CRP level	Median CRP	± Std. Deviation
<37wk (cases)	92	3.65	4.00	2.22
>37 wk (controls)	92	2.76	2.00	1.65
Total	184	3.21	3.00	2.03
P<0.0026; Significant				

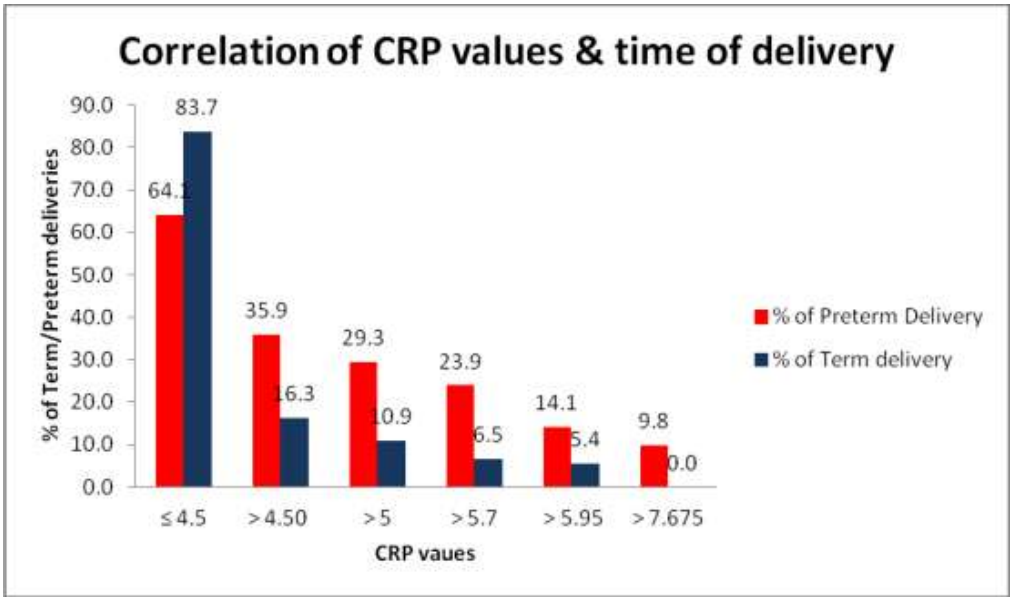

Figure 1: Bar Diagram Showing Correlation of CRP Values & Time of delivery.

Table 2: CRP level > 7 and risk of preterm delivery

CRP levels	<37wk delivery	%	>37 wk delivery	%	Total
>7	11	84.6	2	15.4	13
<7	81	47.4	90	52.6	171
Total	92	50.0	92	50.0	184

P =.0096; Significant; odds risk ratio 6.11; 95%CI=1.31-28.39

Table 3: Association between previous preterm pregnancy and risk of future preterm labour.

CRP levels	<37wk delivery	%	>37 wk delivery	%	Total
Prevspretrm present	24	61.5	15	38.5	39
No prvspretrm	68	46.9	77	53.1	145
Total	92	50.0	92	50.0	184

P=0.107; Not Significant; Odds risk ratio 1.82

Table 4: Association between plasma C-reactive protein concentrations at different thresholds and preterm delivery according to multivariate conditional logistic regression analysis.

Percentiles	Crp Value	No Of Preterm Delivery	%	No Of Term Delivery	%	Multivariate Odds Ratio	Confidence Intervel -95%
<75	<4.5	59	64.13	77	83.70	Referent	na
>75	>4.5	33	35.87	15	16.30	2.84	1.42-5.77
<80	>5	27	29.35	10	10.87	3.52	1.58-7.84
>85	>5.75	22	23.91	6	6.52	4.78	1.82-12.55
<90	>5.95	13	14.13	5	5.43	3.39	1.14-10.04
>95	>7.675	9	9.78	0	00	24.73	1.41-433.24

DISCUSSION

In this study, incidance of preterm pregnancy was about 16.7% which was similar to other studies. Goldenberg RL et al (8) estimated incidence around 12%-13% in USA, while steer p et al 2005,[9] estimated incidence to be 6%-7% of preterm birth. In our study most of preterm labouroccurred inprimigravidas (47.8%) and second gravidas (46.7%). Similar findings were seen in studies by janet m et al and zahrashahshahan et al.,[10] where majority of preterm birth occur in primigravida. In our study about 21.2% pateints had history of previous preterm pregnancy this was in concordance to other studies by Mercer BM et al.,[11] in which women with a previous preterm birth are at higher risk for a recurrence at a rate of 15–50% depending on number of previous events and their timing. However, no significant correlation could be established between previous preterm birth and risk for future preterm birth (P=0.107).

Previously various studies had evaluated maternal concentrations of CRP as an aid to diagnose subclinical infection in pregnant women who experience preterm labor and premature rupture of membranes. In our study mean CRP levels among cases was 3.65 and mean CRP level among control was 2.76 and this differance in mean CRP level was statisticaly significant (p<0.0026). In this

study of singleton pregnancies, we found that very high levels of maternal plasma CRP in early pregnancy were associated with increased risk of preterm delivery. In our study, Compared with women with normal CRP levels, those with CRP level >7 showed statistically significant association between plasma CRP level and preterm delivery (p <.0096; odds ratio 6.11; 95% CI 1.31-28.39).Thus, CRP level>7 identifies a group with higher risk of preterm delivery.The association was stronger for cases who experienced spontaneous preterm delivery versus indicated preterm delivery. These findings suggest that inflammation, as represented by elevated CRP levels, could lead to the physiologic changes that result in preterm delivery. Many studies have evaluated the association between individual or group biochemical marker (s) and preterm birth among asymptomatic women; to date, results of a number of biochemical markers remain inconsistent.

Our results support those from a previous case control study by Hvilsom GB et al,[12] in which serum CRP collected early in the second trimester from 84 women who delivered spontaneously before 37 weeks' gestation and 400 normal controls showed significant association with preterm birth. Dodds WG et al,[13] measured maternal C-reactive protein (CRP) in 109 pregnant women:34 who were in labor before 35 weeks, 25 who were in labor at term and 50 who were not in labor. Maternal CRP of greater than or equal to 0.8 mg/dL identifies a subgroup of women in preterm labor at highest risk of preterm delivery. Tjoa ML et al.,[14] measured CRP levels during the first trimester of pregnancy in 107 women who later developed preeclampsia or gave birth to a growth- restricted baby and found mean CRP levels were significantly elevated in women who later developed preeclampsia (P=0.031) or delivered a growth-restricted baby (P=0.041) when compared with women from the control group, matched for maternal and gestational age, parity, and gravidity. Shahshahan Z et al.,[10] evaluated the relation between C-reactive protein (CRP) with preterm labor and response to tocolytic therapy in Seventy five pregnant women with symptoms of preterm labor (cases) in compare with 75 term women (controls) and suggested that maternal concentrations of CRP can be used as appropriate biomarker for predicting preterm labor and response to tocolytic therapy in pregnant women. Vogel I1 et al.,[15] studied 93 women with symptoms of preterm delivery and found that median sCD163 and CRP levels were significantly higher in women delivering preterm (3.4 mg/L, and 62 nmol/L) compared with the women delivering at term (2.7 mg/L, and <48 nmol/L, Mann-Whitney U test, P < 0.01 and P < 0.001) for sCD163 and CRP, respectively. In another study by Ghezzi F et al.,[16] women who provided blood at 15–18 weeks' gestation, women who delivered spontaneously before 34 weeks had slightly higher median CRP levels (0.6; range, 0–5.6 mg/dl; n ¼ 10) than those who delivered at term (0.5; range, 0–2.6 mg/dl; n ¼ 280)[11] however, the difference was not statistically significant. A recent, small study Torbe A et al 2004,[17] also reported a higher median level of serum CRP in women who subsequently delivered at 36 weeks or less (8.26 mg/liter, n ¼ 30) compared with those who delivered at more than 36 weeks (4.4 mg/liter, n ¼ 35, p < 0.05). However, sample collection age was not determined.

However, results of few studies showed no correlation between CRP levels and preterm birth. Pitiphat W et al.,[18] examined the association of C-reactive protein (CRP), a marker of inflammation, with preterm delivery in 117 women who delivered preterm (< 37 weeks' gestation) and 117 controls (term deliveries) matched on age, race/ethnicity, and smoking status. Median concentration of CRP was 3.2 mg/liter in cases versus 2.4 mg/liter in controls. No significant association was found between quartiles of CRP and preterm delivery. Tarim E et al.,[17] compared women with spontaneous preterm delivery before 37 weeks and women who delivered at term with respect to amniotic fluid C-reactive protein (CRP), glucose levels, and white blood cell counts at the time of genetic amniocentesis and showed that there were no significant differences between the preterm delivery and the term delivery groups with respect to C-reactive protein levels and white blood cell counts. Yoon BH et al.,[20] compared the diagnostic performance of maternal blood C-reactive protein, white blood cell count (WBC), and amniotic fluid (AF) WBC in the identification of positive AF culture, histologic and clinical chorioamnionitis, and neonatal morbidity in women with preterm premature rupture of membranes (PROM). Amniotic fluid WBC performs better than C-reactive protein and maternal blood WBC in the diagnosis of positive AF culture, histologic and clinical chorioamnionitis, and neonatal morbidity in women with preterm PROM.

In the present study, we compared women's CRP levels below the 75th percentile with those whose CRP levels were at or above the 75th, 80th, 85th, 90th, and 95th percentiles to hold the comparison group constant and were able to find a dose-response relation between CRP and preterm delivery for women in the highest quartile. CRP is a proinflammatory marker plays many roles in the inflammatory process by binding to the surface of pathogens and opsonizes them for uptake of phagocytes. It can also activate the classic complement cascade by binding to C1q,[21] Another proinflammatory function of CRP includes the induction of cytokines and tissue factor in monocytes.[22] However, its main function is antiinflammatory by decreasing neutrophil migration to the site of inflammation, preventing adhesion of neutrophils to endothelial cells,[23] and affecting clearance of nuclear antigens released from apoptotic or necrotic cells.[24] Apart from infections, inflammation, and trauma, factors associated with increased levels of CRP include obesity, cigarette smoking, hormone use, metabolic syndrome, and cardiovascular disease.[28] Moderate alcohol consumption, increased physical activity, and medication use (particularly statins, fibrates,

and niacin) are associated with reduced CRP levels.[25] Recent literature identifies adiposity as a key factor in low-grade chronic inflammation.[26] Higher body mass index is associated with elevated CRP concentrations in adult men,[27] nonpregnant women,[27] and pregnant women.[28] In our study, the positive association between CRP and preterm delivery persisted after adjusting for prepregnancy body mass index and other known risk factors for preterm delivery including maternal age, race/ethnicity, and smoking. In tropical country like India, this is the first study that have evaluated associations between biomarkers and adverse pregnancy outcomes in form of preterm birth. The age/weight/parity matched case-control approach improved the efficiency of our study, and the prospective design and good follow up of patients allowed us to evaluate temporality and minimized possible selection bias. In this study, we determined gestational age by last menstrual period and ultrasound to reduce errors. Although presence of genitourinary infections was determined by using International Classification of Diseases codes in medical records but chorioamnionitis which is usually asymptomatic that may precipitate preterm delivery might not be detected.

In summary, we found statistically significant difference between plasma CRP values between case and control and that CRP levels of more than 7 mg/ liter in early pregnancy were significantly associated with risk of subsequent preterm delivery independent of many other determinants of preterm delivery. The association was apparent primarily for spontaneous preterm delivery.

CONCLUSION

To conclude we found that CRP levels of more than 7 mg/liter in early pregnancy were significantly associated with preterm delivery independent of many other determinants of preterm delivery. The association was apparent primarily for spontaneous preterm delivery. These results are consistent with the hypothesis that chronic low-grade inflammation may raise CRP levels and cause preterm delivery. This study will not only help in establishing CRP as biochemical markers associated with preterm birth leading to better understanding on the underlying mechanisms or pathways leading to preterm birth, but also, this valuable tool can guide in designing the most effective targeted intervention strategies aimed at women at risk for preterm birth.

LIMITATION OF STUDY AND RECOMMENDATIONS

Additional research that improves understanding of the mechanisms of preterm birth is important. In addition to the development and validation of multiple biochemical markers to be used independently or in conjunction with other clinical and biophysical markers, demographic, and behavioral risk factors, future work in this area should include further refinement of study design and methodology in the evaluation of gene-gene and gene-environment interaction studies and the role of epigenetics in predicting preterm birth across diverse populations.

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