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PATTERNS OF PLASMA C-REACTIVE PROTEIN LEVELS IN EARLY PREGNANCY AND CONSEQUENT RISKS OF PRETERM DELIVERY

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ABSTRACT: OBJECTIVE(S): To examine the hypothesis correlating the association of raised maternal serum C-reactive protein levels with the increased risk of preterm labour and to identify the role of infections and inflammations in preterm labor. **METHODS/STUDY DESIGN:** The prospective study was conducted in 100 primigravida patients with singleton pregnancies aged between 18-35 years. Their quantitative serum C-reactive protein level was measured at 5-20weeks of gestation according to their enrolment in the antenatal clinic. **RESULTS:** Majority of patients who had serum CRP levels in higher range delivered at preterm. 70% of patients who delivered at preterm had serum CRP levels >7mg/L. None of the patients who had serum CRP levels <2.5mg/L delivered at preterm. 73.3% of preterm patients presented with leaking per vaginum along with other complications, in these patients mean CRP level was 7.6mg/L. **CONCLUSION:** Our Endeavour in this research was to examine a marker (Serum CRP) which is not only easily sampled but also is cost effective especially in our Indian set up. Raised serum CRP concentrations in early pregnancy are associated with increased risk of preterm birth.

KEYWORDS: Early pregnancy, Plasma C-Reactive Protein, Preterm birth.

INTRODUCTION: Currently preterm labour is one of the most challenging, enigmatic problems confronting the obstetrician and perinatologists as this unfortunate episode in the course of women's pregnancy takes a heavy toll of perinatal mortality which accounts for 60-70% of all causes. Systemic maternal infections have been associated with preterm delivery. Intrauterine infections may contribute to 40-50% of all preterm births. 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. Unfortunately most inflammatory markers raised in amniotic fluid and umbilical cord employ an invasive process for their detection. On the other hand, circulating C-reactive protein levels provide an alternate method of detecting women at high risk of preterm delivery. Maternal serum concentrations of CRP levels have been studied as an aid to diagnosing subclinical infections in pregnant women who experience preterm labour and premature rupture of membranes. There have been conflicting reports in previous studies done related to the same topic. So, we tried to examine the association of CRP levels in pregnancy and subsequent risk of preterm delivery among a cohort of 100 singleton pregnant females.

AIMS AND OBJECTIVES:

1. To examine the hypothesis correlating the association of raised maternal serum C-reactive protein levels with the increased risk of preterm labour.
2. To identify the role of infections and inflammations in preterm labour.

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MATERIALS AND METHODS: The present study was conducted in 100 primigravida patients registered in Department of Obstetrics and Gynecology, Panna Dhai Mahila Chikitsalaya attached to R.N.T. Medical College, Udaipur.

Our study comprised of 100 pregnant primigravida women with singleton pregnancies aged between 18-35 years. Their quantitative serum C-reactive protein level was measured at 5-20weeks of gestation according to their enrolment in the antenatal clinic. The study period extended from March '10 to Feb '11. These patients were prospectively followed for their modes of delivery which was correlated with the serum quantitative C-reactive protein levels measured during early pregnancy.

A detailed history was taken regarding name, age, registration number, address, socioeconomic status, educational status and relevant menstrual, past, personal, obstetric, medical history. On admission her LMP, EDD, presenting complaints, labour details, mode of delivery and baby details were noted in all patients.

In general physical examination patients' body mass index, anemia, edema as well as systemic examination was done to rule out any significant finding. Routine investigations like Hb, urine, blood group, USG, fasting blood sugar, ECG, VDRL, TORCH, renal function tests along with serum quantitative C-reactive protein levels in early pregnancy were noted.

Per abdominal and per vaginal examination was done on consequent admission when patients went into labour.

Inclusion Criteria:

1. Primigravida
2. Booked Pregnancy.
3. Patients remembering their last menstrual period and having regular menstrual history.
4. Gestational age between 5-20weeks.
5. Healthy subjects aged between 18-35 years.
6. Patients with BMI between 18-30kg/m².

Exclusion Criteria:

Patients who were with:

1. Multiple pregnancy.
2. Multipara.
3. Bad obstetric history.
4. Gestational age >20weeks at first antenatal visit.
5. Unbooked patients.
6. Patients not knowing their last menstrual period.
7. Patients who were on hormone therapy, statins, fibrates, niacin.
8. Patients with alcohol consumption and cigarette smoking.
9. Patients with medical disorders like metabolic syndromes, cardiovascular problems, diabetes, autoimmune disorders, periodontitis, urinary tract infections, renal and liver disease.
10. Obese patients with body mass index $\geq 30\text{kg/m}^2$.

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CRP Determination: Serum CRP measurement was done by ultrasensitive turbidimetric immunoassay. It was determined using Turbilyte–CRP kit development by Tulip diagnostics (P) Ltd. Principle: Endpoint determination of the concentration of CRP through photometric measurement of turbidity produced by agglutination reaction between antibodies to human CRP present in the human sera. The turbidity is measured at 546 nm wavelength and increase in turbidity corresponds to the CRP levels in the test specimen.

Reagents:

- **R1-Activation buffer:** Ready to use.
- **R2-Latex reagent:** Ready to use uniform suspension of polystyrene latex particles coated with anti-CRP antibody.
- **Calibrator:** A lyophilized preparation of serum equivalent to the stated amount of CRP when hydrated appropriately. It is traceable to World Health Organization International reference standard for human C - reactive protein.
- **Preservative:** 0.1% Sodium Azide in the reagents and CRP Reagent Storage and Stability.
 1. Store the reagent at 2-8°C. Do not freeze.
 2. The reconstituted calibrator is stable for 7 days at 2-8°C and 48 hours at 25-30°C.
 3. The working reagent is prepared by mixing R2 and R1 in the ratio of 1:10.

Incubation Time:

- **A1:** I reading at 10 seconds.
- **A2:** II reading at 2 minutes.

CALCULATIONS:

$$\Delta A = A2 - A1$$

$$\text{Concentration of CRP} = \Delta A (\text{sample}) / \Delta (\text{Calibrated}) \times \text{concentration (S) of calibrator}$$

RESULTS & DISCUSSION: The present study was conducted on 100 primigravida patients registered in the Department of Obstetrics and Gynecology, Panna Dhari Mahila Chikitsalaya attached to RNT Medical College, Udaipur who had their serum CRP levels measured at 5-20 weeks of gestation.

In our study 78% of sample collection was between 10-14 weeks of gestation while in 22% it was between 15-19 weeks. In our study, sample collection before 10 weeks was not possible as patients were mostly registered in antenatal clinic after 10 weeks.

Pitiphat et al.,¹ performed blood sampling between 5.3–19.3 weeks (mean 9.7; SD 2.1).

Lohsoonthorn et al.,² collected the blood samples at an average of 13 weeks of gestation.

In our study out of 15 patients who delivered at preterm 9 (60%) had serum CRP levels >7.5mg/L. while 5 (33.3%) had serum CRP levels 5-7.5mg/L. Only 1 patient (6.6%) had normal serum CRP level. None of the patients having levels <2.5mg/L delivered preterm.

While out of 85 patients, who delivered at term 55 (64.7%) patients had serum CRP levels less than 2.5mg/L. Only 1 patient (1.1%) patient had serum CRP level >7.5mg/L delivered at term.

Our observation correlated with the study conducted by Pitiphat et al.,¹ Median CRP levels were higher in women who delivered before 34 weeks of gestation=5mg/L (2.2-8.2mg/L) than in

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those who delivered between 34 to less than 37 weeks=2.8mg/L (0.9-6.2mg/L). Those who delivered at term had mean CRP level at 2.4mg/L (0.9-1.9mg/L). Compared to pregnant females with normal CRP levels, those with CRP levels ≥ 8 mg/L had a greater than two folds higher odds of preterm delivery. The association was stronger for those who experienced spontaneous preterm delivery versus indicated preterm delivery. But according to the results of the study, overall median concentration of CRP level was 3.2mg/L. in preterm deliveries and 2.4mg/L. in controls. Thus, no significant association was found between quartiles of CRP and preterm delivery.

However, serum CRP levels exceeding the threshold defined in the literature were associated with the increased risk of preterm delivery (odds ratio=2.55, 95% CI: 1.05-6.02 for CRP levels ≥ 8 mg/L).

According to, Lohsoonthorn et al.,² elevations in CRP concentration were associated with the overall risk of preterm delivery. After adjusting for confounding factors, odds ratio for highest quartile (≥ 7.5 vs < 2 mg/L) of CRP was 2.04 (95% CI: 1.13-3.69).

In this study women in the highest quartile as compared with the women in the lowest quartile, experienced a 1.88 fold increased risk of overall preterm delivery (OR=1.88, 95% CI: 1.15-3.09).

They observed 2.04 folds increased risk of preterm delivery among women with CRP concentration of ≥ 7.5 mg/L. as compared with women whose concentrations were < 2 mg/L (OR=2.04, 95% CI: 1.13-3.69).

Hvilsom et al.,³ reported that women with CRP concentrations $\geq$ 85th percentile (≥ 7.6 mg/L) experienced 2 fold increased risk of preterm delivery (OR=2.0, 95% CI: 1.2-3.5) compared with women who had lower CRP concentrations.

Ghezzi et al.,⁴ observed no association between maternal blood CRP concentrations and risk of moderate preterm delivery (34-36 weeks) or very preterm delivery (< 34 weeks).

Vogel et al.,⁵ studied the biomarkers for the prediction of preterm delivery. A likelihood ratio of 5-10 was found for maternal serum CRP levels. Difference in study designs, the timing of blood collections, the underlying characteristics, study populations and incomplete or no control for confounding may account for the variability in the results across studies.

46.7% of preterm deliveries (n=7/15) are on account of PPRM and mean CRP levels are 7.6mg/L while 26.7% (n=4/15) of preterm deliveries which are very preterm also show mean CRP levels in the range of 6.85mg/L. Results of other studies are as follows:

According to Lohsoonthorn et al.,² stratified analysis indicated that elevated serum CRP was associated with increased risk of spontaneous preterm labour (Odds ratio=2.15, 95% CI: 0.85-5.42), medically indicated preterm delivery (Odds ratio=3.29, 95% CI: 0.98-11.02) and very preterm delivery (Or=20.6, 95% CI: 2.53-8.03), but not with preterm premature rupture of membranes (Or=1.48, 95% CI: 0.56-3).

In this study 8.3% of women delivered preterm (Out of which 32.9% were spontaneous preterm deliveries, 37% were preterm PROM, 30% medically indicated preterm delivery. There lies a strong association between medically preterm delivery and serum CRP levels for moderate preterm delivery.

For moderate preterm delivery, there appears no association between it and the maternal serum CRP levels.

However, there appears some association between highest quartile of serum CRP (≥ 7.5 mg/L) and the risk of very preterm delivery.

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Last inferences from these results may be hindered because of the small sample size and extremely wide confidence interval.

Lohsoonthorn et al.,² noted that elevated CRP concentrations (≥ 7.5 mg/dL) were associated with a 2.15 fold increased risk of spontaneous preterm labour (95% CI: 0.85-5.42) and 3.29 fold increased risk of medically indicated preterm delivery (95% CI: 0.98- 11.02) but not with increased risk of premature rupture of membrane preceding preterm delivery (OR = 1.48, 95% CI: 0.56-3.86).

Pitiphat et al.,¹ reported a stronger association among cases who experienced spontaneous preterm delivery (OR=4.64, 95% CI: 0.94-22.96) than medically indicated preterm deliveries. The Incidence of preterm delivery in the study period comes out to be 10.4%. Majority of patients who had serum CRP levels in higher range delivered at preterm. 70% of patients who delivered at preterm had serum CRP levels > 7 mg/L. Thus indicating that if in early pregnancy serum CRP levels are elevated the risk of delivering at preterm is increased.

None of the patients who had serum CRP levels < 2.5 mg/L delivered at preterm. Although 73.3% of preterm patients presented with leaking per vaginam along with other complications, only 46.7% of preterm patients were purely PPRM. In these patients mean CRP level was 7.6mg/L.

26.7% of patients delivered as very preterm and their mean serum CRP level was ~ 6.8 mg/L.

In medically indicated preterm deliveries mean serum CRP level was 8mg/L.

Mean serum CRP level in moderate preterm delivery was lower i.e. 3.5mg/L.

CONCLUSION: Prevention of preterm birth remains an elusive goal. However, it has always been a genuine and novel approach to prevent preterm births in selected high risk groups. For this purpose, we have to gain access to such a marker which helps us to identify such high risk groups. Although molecules like fetal fibronectin, PIGFB1 (Phosphorylated insulin like growth factor binding protein- 1) have revolutionized the present scenario. Their limitations in terms of cost effectiveness and earlier detection persist. So, our endeavour in this research was to examine a marker (Serum CRP) which is not only easily sampled but also is cost effective especially in our Indian set up.

Our study has several strengths. First, we determined CRP levels using serum in early pregnancy so as to negate the false elevated subsequent maternal CRP levels at later stages. Second, we excluded almost all other identifiable causes of preterm births thus focusing on hidden i.e. often undiagnosed cases. Third, our prediction and identification of high risk patients is at a much earlier gestational age. Fourth, sample collection is quite easy, affordable and follow up is practically possible.

Although our study has faced trials and tribulations of time, nevertheless we do not deny of some limitations like single estimation of serum CRP as an index of integrated measurement of maternal inflammatory status during pregnancy.

However, we can emphatically conclude that indeed raised serum CRP concentrations in early pregnancy are associated with increased risk of preterm birth.

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Gestational Age (in weeks)	No. of Patients	Percentage
<10	0	0%
10-14	78	78%
15-19	22	22%
>20	0	0%

Table 1: Distribution of Gestational Age in study population at which serum CRP levels was collected

Serum CRP Levels (mg/L)	Total		Outcome			
			Preterm delivery		Term delivery	
	n=100	%	n = 15	%	n = 85	%
<2.5	55	55%	0	0.00%	55	64.7%
2-5 – 4.9	26	26%	1	6.6%	25	29.4%
5.0 – 7.5	9	9%	5	33.3%	4	4.7%
>7.5	10	10%	9	60%	1	1.1%

Table 2: Distribution of Study Population According to serum CRP Levels

Sl. No.	Type of Preterm Delivery	Mean Serum CRP Levels (mg/L)	Preterm Delivery	
			No. of preterm deliveries n =15	%
1	Very Preterm Delivery	6.8	4	26.7%
2	Mod. Preterm Delivery	3.5	1	6.7%
3	Medically Indicated	8	3	20%
4	PPROM	7.6	7	46.7%

Table 3: Correlation of Mean CRP Levels in subtypes of Preterm Delivery

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