Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/8yva6711

A STUDY ON PATTERNS OF PLASMA C-REACTIVE PROTEIN LEVELS IN EARLY PREGNANCY AND CONSEQUENT RISK OF PRETERM DELIVERY

Dr Duha Qari[1], Dr Anjum Malik[2], Dr Yousha Muneeb Gillani[3*], Dr Ronuma Barooj Qari[4], Dr Syed Faheem Maqbool[5]

[1*]Senior Resident, Department Of Obstetrics And Gynaecology, Skims Maternity Hospital Soura Srinagar
[2]Associate Professor, Department Of Obstetrics And Gynaecology, Skims Medical College Hospital Bemina Srinagar
[3*]Senior Resident, Department Of Anaesthesiology And Critical Care, Government Medical College Srinagar
[4]Speciality Trainee (Year 1), Royal Derby Hospital
[5]Junior Speciality Doctor, Higher University Hospital Birmingham

***Corresponding Author:** Dr Yousha Muneeb Gillani
*Senior Resident, Department Of Anaesthesiology And Critical Care, Government Medical College Srinagar, House No. 5 Al Hijaz Lane, Opposite Police Post Qamarwari, Srinagar, J&K, 190010. Phone No: +917889662580, Email: yousha.gillani@gmail.com

ABSTRACT

Background: Maternal concentrations of C-reactive protein (CRP) has been studied as an aid in diagnosing subclinical infection in pregnant women who experience preterm labour and premature rupture of membranes. Elevated levels of CRP measured during pregnancy have been linked to adverse pregnancy and neonatal outcomes.

Aim: To study the association of serum C–reactive protein levels in early pregnancy with subsequent preterm delivery and to establish it as a predictive tool for preterm labour.

Methods: Maternal plasma C-reactive protein levels were measured in early pregnancy (< 22 weeks gestation) and were correlated with the time of delivery (preterm/term). A value of 3.6mg/l was taken as cut off for CRP positive or negative. Women were followed up till delivery for the outcome and the values were correlated based on the gestational age at delivery.

Results: Association of CRP levels with preterm delivery was found to be significant. 74.4% patients with CRP levels of > 3.6mg/l had preterm delivery against 14.2% who delivered at term whereas only 25.6% delivered preterm with CRP level <3.6mg/l. CRP levels (>3.6mg/l) had acceptable diagnostic accuracy value in preterm delivery (sensitivity-74.3%, specificity 85.8% , PPV-59.2% , NPV-92.4% and accuracy 83.3%)

Conclusion: The present study demonstrates a positive association between elevated CRP in early pregnancy and the subsequent risk of preterm delivery, which can serve in improving pregnancy management and neonatal outcome.

KEYWORDS: Plasma C Reactive Protein, Preterm Delivery, Early Pregnancy.

ABBREVIATIONS

CRP: C Reactive Protein, CI: Confidence Interval, ICU: Intensive Care Unit, LSCS: Lower Segment Caesarean Section, PID: Pelvic Inflammatory Disease, PPROM: Preterm Premature Rupture Of Membranes, PTD: Preterm Delivery.

MAIN TEXT

Introduction: Frequency of preterm birth which is also known as childbirth at less than 259 days of gestation or 37 completed weeks since the first day of the women's last menstrual period is from 5% to 13% in high income countries and the incidence is increasing [**1, 2].** It is the leading cause of neonatal morbidity and mortality worldwide and accounts for 75% of neonatal deaths and 50% of long-term morbidity including respiratory disease and neurodevelopment impairment [**3]**. Globally, an estimated 15 million babies are born prematurely each year, representing 11.1% of all live births. One million of them die from the complications of preterm birth [**4].**

Nearly 40%-50% of preterm births occur following spontaneous labour, 30% due to preterm premature rupture of membranes (pPROM) and remaining 30% are iatrogenic terminations for maternal or fetal benefit [**5]**. Known causes of spontaneous preterm labour include infection (intrauterine or extrauterine), multiple gestation, placental abruption, hormonal disruptions and other factors, though a large proportion of preterm births are idiopathic [**6]**. As many as 50% of spontaneous preterm births may be associated with infection [**7]**. 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 concentration of pro inflammatory cytokines such as interleukin-6 and interleukin-8 in serum have been reported in women with symptoms of preterm labour and have been prospectively associated with preterm birth **[8]**

Maternal concentrations of C-reactive protein (CRP) have been studied as an aid in diagnosing subclinical infection in pregnant women who experience preterm labor and premature rupture of membranes **[9,10].** Recently, elevated levels of CRP measured during gestation have been linked to adverse pregnancy outcomes such as preeclampsia **[11, 12]** and intrauterine growth restriction **[12].** CRP is an easily detectable serological marker **[13] and** is primarily produced in the hepatocytes in response to cytokine released from the inflammatory site **[10].** Forty-eight hours after stimulation the acute phase response reaches its' maximum and a reliable measurement can be made from blood sample **[13].** The median normal concentration of C-reactive protein is 0.8 mg/L, with 90% of apparently healthy individuals having a value less than 3mg/L and 99% less than 12mg/L. Higher values are abnormal and indicate the presence of organic disease **[14].** Measuring inflammatory markers can be a predictive method for detecting women at high risk for preterm labor.

According to previous studies, increased levels of serum CRP have a relationship with intrauterine infection**. [15,16**] There are studies at hand on CRP levels as potential means for identifying hidden infection in pregnant women who have experienced PTB and PPROM**[17].** Tillett WS et al. were the first to isolate CRP in 1930 as a substance in the serum of patients with acute inflammation **[18].**

The studies on relation between CRP levels in maternal circulation during the second trimester of pregnancy and risk of pre-term delivery showed contradictory results.

Hvilsom GB et al. **[19**] reported a significant association of elevated serum CRP levels with a nearly twofold increased risk of delivery before 37 weeks' gestation. Ghezzi F et al. found no relation between circulating CRP levels and preterm delivery. However, they observed a higher median amniotic fluid CRP level in women who delivered preterm compared with women who

delivered at term. Endothelial dysfunction has been postulated to be part of an exaggerated maternal inflammatory response to pregnancy **[20].**

In addition, cross-sectional analyses indicate that CRP is strongly correlated with markers of endothelial activation and dysfunction. Collectively, these findings suggest that C-reactive protein, a marker of systemic inflammation, may be involved in the pathogenesis of preterm delivery.
In light of conflicting reports, we examined prospectively the association between maternal plasma CRP levels in early pregnancy and risk of subsequent preterm delivery among a cohort of singleton pregnant women.

Aim of the study
1) To study the association of serum C–reactive protein levels in early pregnancy with subsequent preterm delivery.
2) To establish serum C–reactive protein levels as a predictive tool for preterm labour, so as to reduce the morbidity and mortality resulting in preterm delivery.

Materials and methods
The present prospective nested case control study was conducted in the Department of Obstetrics and Gynecology, Sher-I-Kashmir Institute of Medical Sciences, Medical College and Hospital, Bemina, Srinagar over a period of 2 years from 2020 to 2022. A total of 180 antenatal women with singleton pregnancy of 12-22 weeks of gestation were enrolled in the study and followed till delivery. Maternal C-reactive protein levels were taken in early pregnancy and were correlated with the time of delivery (term/preterm). The research protocol was approved by the Institutional Ethical Committee.
Patients fulfilling the selection criteria and consented to participate were included in the study.

Inclusion Criteria
- Patients attending Out Patient Department/Emergency unit of Department of Obstetrics and Gynecology for prenatal check-up with gestational age less than 22 completed weeks.
- Patients with a singleton pregnancy.

Exclusion Criteria
- Multiple pregnancy
- Patients with uterine anomalies such as cervical incompetence, malformations of uterus etc.
- Gestational age >22 weeks at initial prenatal visit.
- Pregnant women with H/O smoking, diabetes mellitus, chronic hypertension, cardiac disease, liver disease, lung disease,
- Antiphospholipid syndrome and rheumatoid arthritis were excluded from the study.
- Patients who have conceived after intake of ovulation induction drugs or in vitro fertilization were excluded from this study

A total of 180 pregnant women were recruited for study and were followed till delivery (preterm/term). Maternal plasma C-reactive protein levels were measured in early pregnancy (<22 weeks gestation) and were correlated with the time of delivery (preterm/term).
At the initial prenatal visit, plasma sample was drawn from each participant, collected in ethylenediamine-tetraacetic acid, refrigerated for several hours and then transported to the laboratory where it was centrifuged and frozen until the time of analysis. Gestational age at blood sampling was 12-22 weeks. C-reactive protein levels were measured in the Biochemistry Laboratory of SKIMS, MCH, Bemina, Srinagar with a validated high sensitivity immunoturbidimetric assay on the Siemens Fully Automated Biochemistry Analyser. A value of

3.6mg/l was taken as cut off for CRP positive or negative. Women were followed up to delivery for the outcome and the values were correlated based on the gestational age at delivery.

Results

TABLE 1: AGE DISTRIBUTION OF STUDY PATIENTS IN TWO GROUPS					
AGE (YEARS)	**CASES**		**CONTROLS**		**P-VALUE**
	NO.	**%**	**NO.**	**%**	
≤ 20	2	5.12	9	6.38	0.573
21-34	25	64.10	101	71.63	
≥35	12	30.76	31	21.98	
TOTAL	39	100	141	100	
MEAN±SD (RANGE)	27.5±4.12 (19-36)		27.9±3.59 (18-37)		

Most common age group affected in both the study groups were aged between 21-34 years viz. 64.10% (n=25) in cases and 71.63 (n=101) controls with a mean age of 27.5+4.12 years and 27.9+3.59 years in cases and control group respectively.

TABLE 2: GESTATIONAL AGE AT SAMPLE COLLECTION OF CRP IN TWO GROUPS				
GESTATIONAL AGE (WEEKS)	**CASES**		**CONTROLS**	
	NO.	**%AGE**	**NO.**	**%AGE**
12-15 WEEKS	8	20.5	26	18.4
15-18 WEEKS	27	69.2	105	74.5
18-22 WEEKS	4	10.3	10	7.1
TOTAL	39	100	141	100
MEAN±SD	16.4±1.12		16.3±1.14	

Mean gestational age was 16.4+1.12 weeks and 16.3+1.14 weeks in Cases and Controls, respectively. Majority of the women in both the study groups had 15-18 weeks of gestation at the time of sample collection of CRP with 27 (69.2%) women in Cases and 105 (74.5%) women in Controls.

TABLE 3: COMPARISON BASED ON C-REACTIVE PROTEIN (CRP) LEVELS IN TWO GROUPS

CRP LEVELS	N	MEAN	SD	95% CI	P-VALUE
CASES	39	5.4	2.8	4.52-6.27	<0.001*
CONTROLS	141	2.3	1.1	2.11-2.48	

Mean CRP level was 5.4±2.8 in Cases and 2.3+1.1 in Controls with a statistically significant difference ($p < 0.001$).

TABLE 4: DIAGNOSTIC ACCURACY OF C-REACTIVE PROTEIN (CRP) LEVELS IN PREDICTING PRETERM LABOUR

PARAMETER	VALUE	95% CI
OPTIMAL CUTOFF	≥ 3.6MG/L	-
SENSITIVITY	74.3	58.9-85.4
SPECIFICITY	85.8	79.1-90.6
PPV	59.2	45.3-71.8
NPV	92.4	86.5-95.8
ACCURACY	83.3	77.2-88.1
AREA UNDER THE ROC CURVE	0.952	0.915-0.978

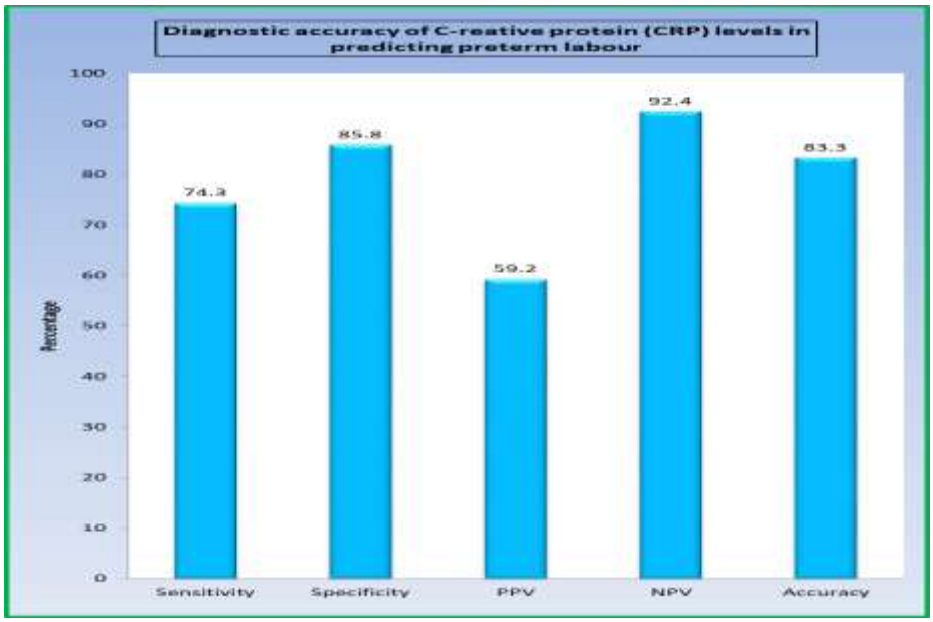

FIGURE 1

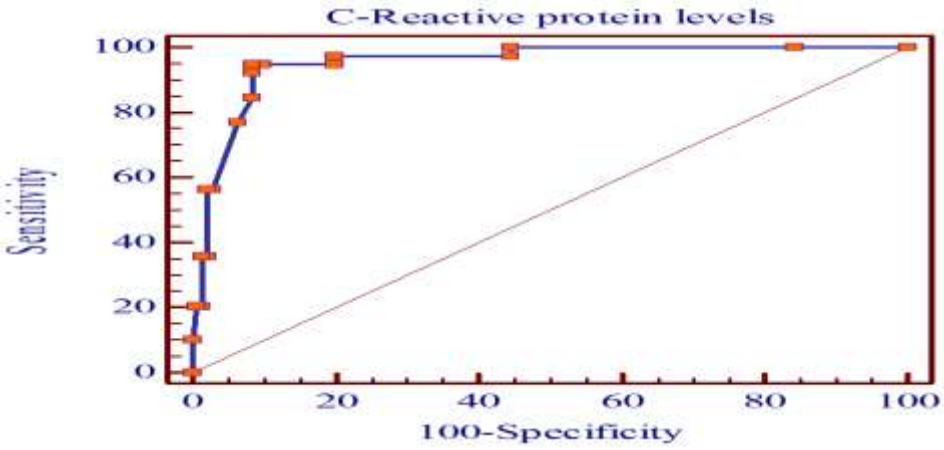

FIGURE 2

By using receiver operating characteristic (ROC) curve, serum CRP levels (Optimal cut-off point >6 mg/l, area 0.952) had acceptable diagnostic accuracy value in distinguishing preterm delivery [(sensitivity (74.3%), specificity (85.8%), positive predictive value (59.2%), negative predictive value (92.4%), accuracy (83.3%)].

TABLE 5: ASSOCIATION OF C-REACTIVE PROTEIN (CRP) LEVELS WITH SUBSEQUENT PRETERM LABOUR IN STUDY PATIENTS

CRP LEVELS	CASES (PRETERM)		CONTROLS (TERM)		P-VALUE
	NO.	%AGE	NO.	%AGE	
≥ 3.6MG/L	29	74.4	20	14.2	<0.001*
<3.6MG/L	10	25.6	121	85.8	
TOTAL	39	100	141	100	

Association of C-reactive protein (CRP) levels with preterm was found to be significant, 74.4% preterm women with CRP levels of > 3.6mg/l against 14.2% term women. CRP levels were <3.6mg/l 25.6% preterm women and 85.8% term women. The mean CRP level observed in cases was higher than the cut-off taken in our study (3.6 mg/l) which demonstrates a positive association between elevated CRP in early pregnancy and the subsequent risk of preterm delivery.

Discussion

Preterm birth is a leading cause of perinatal morbidity and mortality worldwide associated with death of infants. Therefore, prediction and prevention of preterm delivery is very important for improvement in neonatal outcome. Due to recent advancements and improvements in available resources a number of clinical and biochemical markers like C-reactive protein, fetal fibronectin, interleukin-8, alphafetoprotein, insulin like growth factor binding protein-1, granulocyte elastase, measurement of cervical length and detection of cervical funnelling etc. have been utilisedfor the

prediction of preterm delivery. Among these, elevated levels of maternal plasma C-reactive protein are significantly correlated with an increased risk of preterm delivery **[19, 21].** 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 addition to this elevated levels of CRP measured during early 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 **[22].** This association between elevated plasma C-reactive protein and preterm delivery was highlighted by published results of various authors like by **Hvisom GB et al. (2002) [19], Ghezzi F et al. (2002) [23], Halder A et al. (2013) [24] and Kulshreshtha S et al., (2015) [25].** In present study we also achieved comparable results with regard to the effectiveness of maternal plasma C-reactive protein levels in predicting preterm delivery; hence, strengthening the association further.

In our study, most common age group affected in both cases and controls were aged between 25-29 years viz. 46.2% (n=18) in cases and 49.6% (n=70) in controls with a mean age of 27.5+4.12 years and 27.9+3.59 years in Cases and Controls respectively. There was no significant statistical difference in age between cases and controls. **Detho S et al., (2022) [26]** did a similar study in which a total of 88 pregnant women with preterm delivery were included with most of the women being 26-30 years of age with a mean age of 28.26±4.02 years. **Afzal A et al., (2017) [27]** also confirmed that the most common age group affected in their study was 20-34 years among both cases (n=27, 65.9%) and controls (n=115, 72.3%). Mean age of the patients was found to be 28.6±6.7 years and 28.9±5.3 years among cases and controls respectively.

In this study, the plasma CRP levels among the cases and controls were compared. Mean CRP level was 5.4±2.8 in Cases and 2.3+1.1 in Controls with a statistically significant difference ($p < 0.001$). **Pitiphat W et al., (2005) [28],** in their study of 234 patients, found that median concentration of plasma CRP was higher in women who delivered before 34 weeks' gestation (5.0 mg/liter; interquartile range, 2.2–8.2) than in those who delivered between 34 and less than 37 weeks (2.8 mg/liter; interquartile range, 0.9–6.2) and those who delivered at term (2.4 mg/liter; interquartile range, 0.9–4.9). **Lohsoonthorn V et al. (2007) [29]** in their study found a median CRP level of 4.4 mg/L and 3.9 mg/L in cases and controls respectively.

By using receiver operating characteristic (ROC) curve, serum CRP levels (optimal cut off point >3.6 mg/l, area 0.952) had acceptable diagnostic accuracy value in distinguishing preterm delivery [(sensitivity (74.3%), specificity (85.8%), positive predictive value (59.2%), negative predictive value (92.4%), accuracy (83.3%)].

Our results are consistent with the study done by **Sunagawa S et al., (2008) [30].** In their study of 119 patients, they found the sensitivity, specificity, positive predictive value and negative predictive value of CRP in prediction of preterm delivery as 50%, 85.7%, 55.6% and 82.8% respectively. Comparable results were achieved in a study conducted by **Afzal A et al., (2017) [27]** including 200 study subjects where mean CRP level was 5.2±2.9 mg/L in cases and 2.4±1.2 mg/L in controls. Median CRP concentration was also higher in cases (4.8 mg/L) than in controls (2.0 mg/L). The sensitivity, specificity, PPV and NPV of CRP in predicting preterm delivery were found to be 61.0%, 88.7%, 58.1% and 89.8% respectively, at a cut-off value of 3.6 mg/L. In the study done by **Mazor M et al., (1993) [15]** the sensitivity, specificity, positive predictive value and negative predictive value of CRP in predicting preterm delivery due to intra-amniotic infection were found to be 71.5%, 73.2%, 31.3% and 93.8% respectively.

This study tried to establish association between C-reactive protein (CRP) levels with preterm delivery and the data obtained was found to be statistically significant ($p < 0.001$). Among the cases who delivered preterm 74.4% subjects had elevated CRP levels of >3.6mg/l against 14.2% subjects with elevated CRP who delivered at term. CRP levels were <3.6mg/l in 25.6% study subjects who delivered preterm against 85.8% women who delivered at term. **Kulshreshtha S et al., (2015) [25]** in their study mention that 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.
Our study has certain limitations. Precision of estimates was affected by the relatively small number of cases who delivered preterm, especially those women with extremely high CRP values. As in other observational studies, we cannot rule out the possibility of residual confounding. In this study, we were unable to evaluate whether CRP levels were elevated before as well as after conception. We also do not know whether the association of CRP with pre-term delivery reflects causality; that is, if reducing CRP levels would result in less preterm delivery.

Conclusion

A high C-reactive protein level at the beginning of a pregnancy is associated with a nearly two fold increased risk of preterm delivery. The present study demonstrates a positive association between elevated CRP in early pregnancy and the subsequent risk of preterm delivery. An optimal cut off point ≥3.6mg/l was taken which showed an acceptable diagnostic accuracy value.

Conflict of Interest: Nil

REFERENCES

1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371:75–84.
2. Roberts CL, Morris JM, Rickard KR, Giles WB, Simpson JM, Kotsiou G, et al. Protocol for a randomised controlled trial oftreatment of asymptomatic candidiasis for the prevention of preterm birth [ACTRN12610000607077]. BMC Pregnancy Childbirth. 2011;11:19.
3. Goldenberg RL, Culhane JF,Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75-84.
4. Howson CP, Kinney MV, Lawn JE. Born too soon: The Global Action report on preterm Birth. Geneva, Switzerland: World healthOrganization; 2012.
5. Neonatal and infant mortality. Situation and trends. www.who.int/gho/child_health/mortality/neonatal_infant_text/en/index.html.
6. Lockwood CJ. The diagnosis of preterm labor and the prediction of preterm delivery. Clin Obstet Gynecol. 1995;38:675–87.
7. Goffinet F. Primary predictors of preterm labour. BJOG 2005; 112 (Suppl 1): 38-47.
8. Goldenberg RL, Hauth JC and Andrews WW. Intrauterine infection and preterm delivery. N. Engl. J. Med, 2000;342: 1500-7.
9. Dodds WG, Iams JD. Maternal C-reactive protein and pretermbirth. J Reprod Med 1987; 32: 527–30.
10. Yoon BH, Jun JK, Park KH, Syn HC, Gomez R, Romero R. SerumC-reactive protein, white blood cell count and amniotic fluid white blood cell count in women with premature rupture of membranes. Obstet Gynecol 1996; 88: 1034–40.
11. Wolf M, Kettyle E, Sandler L, et al. Obesity and preeclampsia: the potential role of inflammation. Obstet Gynecol 2001;98:757–62.
12. Tjoa ML, van Vugt JM, Go AT, et al. Elevated C-reactive protein levels during first trimester of pregnancy are indicative of preeclampsia and intrauterine growth restriction. J Reprod Immunol.2003;59:29–37.
13. Bek KM, Nielsen FR, Qvist I, Rasmussen PE, Tobiassen M. C-reactive protein (CRP) and pregnancy. An early indicator of chorioamnionitis. A review. Eur J Obstet Gynecol Reprod Biol 1990; 35: 29–33.
14. Reeves G. C-reactive protein. Aust Prescr 2007;30(3):74-6.
15. Mazor M, Kassis A, Horowitz S et al. Relationship between C- reactive protein-levels and intraamniotic infection in women with preterm birth. J Reprod Med 1993; 38: 799–03.
16. Potkul RK, Moawad AH, Ponto KL. The association of subclinical infection with preterm birth: The role of C-reactive protein. Am J Obstet Gynecol 1985; 153: 642–45.

17. Dodds WG, Iams JD. Maternal C-reactive protein and pretermbirth. J Reprod Med 1987; 32: 527–30.
18. Tillett WS, Francis T Jr. Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus. J Exp Med 1930; 52: 561–71
19. Hvilsom GB, Thorsen P, Jeune B, et al. C-reactive protein: a serological marker for preterm delivery? Acta Obstet Gynecol Scand 2002;81:424–9.
20. Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol 1999; 180(2 Pt 1):499–506.
21. Foulon W, Van Liedekerke D, Demanet C, et al. Markers of infection and their relationship to preterm delivery. Am J Perinatol 1995;12(3):208-11.
22. Cunningham FG, Gant NF, Leveno KJ, Gilstrap LC III, Hauth JC, Wenstrom KD, eds. William Obstetrics, 21st edn. New York: McGraw-Hill, 2001.
23. Ghezzi F, Franchi M, Raio L, et al. Elevated amniotic fluid C- Reactive Protein at the time of genetic amniocentesis is a markerfor preterm delivery Obstet. Gynecol., 2002;186:268-73.
24. Halder A, Agarwal R, Sharma S, Agarwal S. Predictive significance of C reactive protein in spontaneous preterm delivery: a prospective cohort study. Int J Reprod Contracept Obstet Gynecol 2013;2:47-51.
25. Kulshreshtha S, Anjum S, Agrawal A, et al. Patterns of plasma C- reactive protein levels in early pregnancy and consequent risks of preterm delivery. Journal of Evolution of Medical and Dental Sciences 2015; 4(65): 11335-41.
26. Detho S, Habib A, Aziz S, Naz U, Hira AK, Kazi S. Frequency of Elevated Levels of Serum CRP in Patients with Preterm Labor in a Tertiary Care Hospital, Karachi. PJMHS 2022; 16(04): 589-91.
27. Afzal A, Ashraf S, Ashraf M. Maternal plasma C-reactive protein: A predictive tool for preterm delivery. J. Evolution Med. Dent. Sci.2017; 6(5):369-374.
28. Pitiphat W, Gillman MW, Joshipura KJ, Williams PL, Douglass CW and Rich-Edwards JW. Plasma C-Reactive Protein in Early Pregnancy and Preterm Delivery. Am J Epidemiol. 2005; 162(11): 1108–13.
29. Lohsoonthorn V, Qiu C, Williams MA. Maternal serum C-reactive protein concentrations in early pregnancy and subsequent risk of preterm delivery. Clinical Biochemistry 2007;40:330–35.
30. Sunagawa S, Takagi K, Ono K, Miyachi K, Kikuchi A. Comparison of biochemical markers and cervical length for predicting preterm delivery. J Obstet Gynaecol Res. 2008; 34(5): 812-9.