

C-Reactive Protein Level as a Diagnostic Tool for Assessment of Adverse Pregnancy Outcomes in Females with Periodontal Disease – A Randomized Clinical Trial

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Abstract:- Background: An acute-phase protein found in plasma that reflects a measure of acute phase response to inflammation is C-reactive protein (CRP). Early detection of periodontal disease and its prediction can be used by CRP. There is an association of C-reactive protein with adverse pregnancy outcomes, including pre-eclampsia intrauterine growth restrictions, and preterm delivery. The present study endeavor to evaluate serum C-reactive protein level in pregnant women with and without periodontal disease and to compare incidence of pre-term delivery in pregnant women with and without periodontal disease. **Material and methods** This study was undertaken in 40 pregnant women, divided into 2 equal groups of 20 pregnant women within 22nd weeks of gestation; group I (study group) with periodontal disease and group II (control group) without periodontal disease. Blood samples were taken for estimation of C-reactive protein levels from both the groups within 22nd weeks of gestation and were

sent for laboratory analysis. CRP levels were determined using Immuno Turbidometry method on an autoanalyzer. **Result** It was found that the mean value of CRP levels is correlated with high incidence of preterm delivery. Subjects with preterm delivery have high mean CRP values as compared to subjects with normal delivery. **Conclusion** Increased CRP levels in early pregnancy is associated with periodontal disease in pregnant women. In healthy normal controls the incidence of preterm delivery is lesser comparative to pregnant women with periodontal disease.

Keywords: C-Reactive Protein, Periodontal Disease, Preterm.

I. INTRODUCTION

C-reactive protein (CRP) is 120,000-140,000 molecular weight pentameric protein. Tillett and Francis in 1930 discovered that, substance in the serum of patients exhibiting acute inflammation, was found reacting with pneumococcus capsular polysaccharide.^[1] CRP is an acute phase reactant synthesized by liver in response to the inflammatory cytokines like interleukin (IL)-6, IL-1, and tumor necrosis factor-alpha.^[2] Systemic inflammation and periodontal diseases are indicated by circulating C-reactive protein^{[3],[4]} There is a proportional increase of inflammation with increase in high sensitivity CRP levels^[5] therefore the prediction and early detection of periodontal disease is possible by the use of CRP.^[6]

Increased risk of preterm low birth weight,^[7] low birth weight,^[8] and preterm birth^[9] has been associated with periodontal disease. Therefore, C-reactive protein level can be used as a diagnostic tool as a plausible mediator of association between periodontitis and adverse pregnancy outcomes.^[10]

Thus the aim and objective of the present study were to assess serum C-reactive protein level in pregnant women with and without periodontal disease and to compare incidences of preterm delivery.

II. MATERIAL AND METHODS

Study was conducted in the Department of Obstetrics and Gynaecology, Kamla Nehru state hospital for Mother and Child, Shimla and at Civil Hospital, Sundernagar, Himachal Pradesh.

Patients who participated in the study are of age group between 18-34 years, systemic healthy, Singleton pregnancy, pregnant females within 22nd week of gestation were included. History of antibiotic intake during pregnancy, periodontal therapy in the past six months, other risk factors like alcohol consumption, smoking or drug use, multiple pregnancy, pregestational diabetes, previously diagnosed uterine anomalies, patients with medical history of cardiac disease and chronic renal disease, without access for communication and correspondence address, having less than 18 teeth were excluded.

The 40 subjects who met the selection criteria were divided into study group (Group I) and the control group (Group II). A questionnaire was administered by the examiner. Information was obtained regarding socioeconomic status of the patients from this questionnaire. Modified Kuppuswamy's socioeconomic scale^[11] was utilized in this study for the stratification of the patients. Five classes were stratified comprising of upper, upper-middle, lower-middle, upper-lower and lower.

An Informed consent was obtained from all the patients.

All the patients were followed through a post partum visit to evaluate the incidence of preterm delivery.

❖ *Laboratory analysis*

Estimation of C-reactive protein levels from both the groups within 22nd weeks of gestation were done and sent for laboratory analysis after blood samples were taken. CRP levels were determined using Immuno Turbidometry method on an autoanalyzer. The detection limit of the test was 2.9 mg/L and the biological reference interval was <5 mg/L.

❖ *Measurement of periodontal status*

➤ *Gingival inflammation*^[12]

Loe & Silness Gingival Index was used to know the severity of gingivitis

➤ *Probing depth*

The probing depth measurement was assessed by means of University of Michigan "O" probe with Williams's markings. Except third molars probing depth measurements were made at the mesio-buccal, buccal, disto-buccal, disto-lingual, lingual, and mesio-lingual positions of every tooth.

➤ *Clinical attachment level*

The distance between the base of the pocket and a fixed point on the crown, such as the cemento-enamel junction (CEJ) was made to measure the clinical attachment level. Except third molars Clinical attachment level measurements were made at the mesio-buccal, buccal, disto-buccal, disto-lingual, lingual, and mesio-lingual positions of every tooth.

➤ *Community periodontal index of treatment needs (cpitn)*^{[13],[14]}

It was noted using a CPITN probe. The ten specified index teeth were recorded. These teeth have been identified as the best estimators of the worst periodontal condition of the mouth and only one score, the highest is recorded.

The obtained results were compiled and subjected to statistical analysis using SPSS software by a qualified statistician.

III. RESULTS

In this study, an attempt was made to evaluate the CRP levels in pregnant women with and without periodontal disease in subjects within a defined age group and similar body mass index. In group I (Study) the mean age of the subjects was 24.8±4.66 years. In group II (Control) the mean age was 25.3±2.29 years. In group I (Study) mean BMI of the subjects was 20.70±2.95 kg/m². In group II (Control) the mean BMI was 20.86±2.95 kg/m². There was a difference in mean age of subjects in both the groups which were statistically insignificant (p=0.67, p>0.05). The difference in mean BMI in

the group I (Study) and the group II (Control) was statistically insignificant (p=0.86, p>0.05) (Table 1).

Table 1 Comparison of age and body mass index between group I (Study) and group II (Control).

Variable	Groups	N	Mean	SD	Std. Error Mean
Age	Cases	20	24.8	4.66	1.04
	Controls	20	25.3	2.29	0.51
BMI (Kg/m ²)	Cases	20	20.70	2.95	0.66
	Controls	20	20.87	2.95	0.66
Age: p=0.67, p>0.05 BMI: p=0.86, p>0.05					

The majority of subjects enrolled in the study were from upper middle, 18 (45.0%), followed by lower middle, 14(35.0%), upper lower, 7(17.50%) and 1(2.5%) from upper class. There was statistically significant (p=0.024, p<0.05) with the difference in socioeconomic status between group I (Study) and group II (Control) (Table 2).

Table 2: Comparison of socioeconomic status between group I (Study) and group II (Control)

Socioeconomic status	Cases N (%)	Controls N (%)	Total N (%)
Upper	1 (5.0%)	0 (0.0%)	1 (2.5%)
Upper middle	5 (25.0%)	13 (65.0%)	18 (45.0%)
Lower middle	9 (45.0%)	5 (25.0%)	14 (35.0%)
Upper lower	5 (25.0%)	2 (10.0%)	7 (17.5%)
Total	20 (100.0%)	20 (100.0%)	40 (100.0%)
$\chi^2 = 14.6$ & p=0.024, p<0.05			

The incidence of preterm and term delivery in group I (Study) was 11(55.0%) and 9 (45.0%) respectively. The incidence of preterm and term delivery in group II (controls) was 3(15.0%) and 17(85.0%) respectively. A statistically significant difference was found in incidence of preterm and term delivery in the Study and Control group. The incidence of preterm delivery between group I (Study) and group II (Control) was statistically significant (p=0.033, p<0.05). The incidence of term delivery between group I (Study) and group II (Control) was statistically significant (p=0.05) (Table 3).

Table 3: The incidence of preterm and term delivery between group I (Study) and group II (Control).

Incidence	Cases N (%)	Controls N (%)	Total N (%)	χ^2	P value
Number of preterm% of Total	11 (55.0)	3 (15.0)	14 (35.0)	4.57	0.033**
Number of term % of Total	9 (45.0)	17 (85.0)	26 (65.0)	3.84	.05**
Total number % of Total	20 (50.0)	20 (50.0)	40 (100.0)		

** Significant at 0.05 level of significance

The mean value of C-reactive protein level in group I (Study) was 13.2±10.0 mg/L. The mean value of C-reactive protein level in group II (Control) was 5.5±4.2 mg/L. The difference in mean CRP level was statistically significant (p=0.002, p<0.05) (figure 1).

The mean value of C-reactive protein level of group I (Study) with normal (Term) delivery was 4.94±3.24 mg/L. The mean value of C-reactive protein level of group I (Study) with preterm delivery was 20.02±8.32 mg/L. The difference in mean value of CRP level in term and preterm delivery in group I (Study) was found to be statistically significant (p=0.00, p<0.01) (Table 4).

Table 4: Comparison of mean C-reactive protein level (mg/L) in subjects of group I (Study) with normal (Term) and preterm delivery (Unpaired t-test).

Cases	N	Mean	SD	Std. Error Mean	t	df	P value
CRP level in term delivery	9	4.94	3.24	1.08	5.11	18	0.00*
CRP level in preterm delivery	11	20.02	8.32	2.51			

* Significant at 0.01 level.

The mean value of C-reactive protein level of group II (Control) with normal (Term) delivery was 4.85 ± 3.07 mg/L. The mean value of C-reactive protein level of group II (Control) with preterm delivery was 8.23 ± 9.97 mg/L. The difference in mean value of CRP level in term and preterm delivery in group II (Control) was found to be statistically significant ($p=0.03$, $p<0.05$) (Table 5).

Table 5: Comparison of mean C-reactive protein level (mg/L) in subjects of group II (Control) with normal (Term) and preterm delivery (Unpaired t-test).

Controls	N	Mean	SD	Std. Error Mean	t	df	P value
CRP level in term delivery	17	4.85	3.07	0.74	2.23	18	0.03**
CRP level in preterm delivery	3	8.23	9.97	5.10			

** Significant at 0.05

The results of this cohort study demonstrate the elevated CRP levels in pregnant women with periodontal disease compared to healthy controls and preterm delivery rate is higher in women with periodontal disease compared to control group.

IV. DISCUSSION

Even though there is advancement in obstetrical prevention, diagnostics, and therapy preterm birth (birth before 37 weeks of gestation) is still considered to be a major problem and remains the leading cause of morbidity and mortality [15]. An effort was made to assess the CRP levels in pregnant women with and without periodontal disease in this study. The elevated CRP levels in pregnant women with periodontal disease compared to healthy controls and preterm delivery rate is higher in women with periodontal disease compared to control group as a result of this cohort study.

The potential risk factors for preterm low birth weight are estimated at the age less than 17 and more than 34. However the subjects who participated in the present study are between 19-34 years of age, the mean age being 24.8 years, yielding no significant difference in mean ages in both the groups. Mean age of the present study is compared with the mean age of subjects in studies done by **Offenbacher et al** [7], **Hasegawa et al** [16] and **Dongming et al** [17]. The difference in mean BMI in both the groups was statistically insignificant ($p>0.05$). There was a statistically insignificant relationship between preterm birth and body mass index between the present study as well as the study done by **Hasegawa et al** [16].

The difference in mean socioeconomic status between the cases and the controls is found to be statistically significant ($p<0.05$). According to the study done by **Radnai et al** [18] the data showed insignificant difference as the preterm birth was more common amongst women who were less educated and had lower social status. Delivering a premature, low weight baby increases fourfold increase of periodontal disease prevalence, regardless of the control of other risk factors such as age, smoking, and social extraction according

to **Mokeem et al** [19], **Cruz et al** [20]. There was a positive association between periodontal disease and low birth weight. The correlation between periodontitis and PTB/LBW were reported in several cross sectional studies. According to study which was published in 2016 where women with PTB were found to have worse periodontal parameters [21]. PTB is a multifactorial condition and that periodontitis and the presence of periodontal pathogens are not sufficient to trigger PTB which was suggested in the year 2016 by **Martinez- Martinez et al.** [22]. CRP levels exceeding the threshold that are associated with increased risk of preterm delivery. [23] In the present study, the mean value of reported CRP levels was correlated with high incidence of preterm delivery. Subjects with preterm delivery have high mean CRP values as compared to subjects with normal delivery.

In this study, the association observed between CRP level and periodontal disease in pregnancy may or may not be causal. Periodontal infection and inflammation may cause elevated CRP levels. CRP amplifies the inflammatory response through complement activation, tissue damage, and induction of inflammatory cytokines in monocytes [2] and therefore may mediate the association between periodontitis and adverse pregnancy outcomes.

This raises the possibility that CRP may mediate association between periodontal disease and preterm delivery. However, during the interpretation of data one has to be cautioned, as there is multi-factorial etiology of both periodontal disease and preterm delivery, in addition the role of confounding effect can't be completely excluded.

Our present study had several strengths. First, as the relationship between multiple gestations and preterm labor is well established subjects with only singleton gestation were included. [24] Second, there is a free access to all the participants in medical and prenatal medical care. Third, the temporal relationship between elevated maternal serum CRP concentrations and subsequent risk of preterm delivery was determined by CRP status using serum collected in early pregnancy. However, our study also had some limitations. First, based on the social factors the subjects in our study were

relatively non-homogenous. Second, a single measurement of serum CRP is not likely to provide a time-integrated measure of maternal inflammation status during the index pregnancy. Third, a relatively small number of subjects were included in the study.

At the end of the study the association of CRP with preterm delivery couldn't be established conclusively. Future studies on the maternal and fetal immune response to chronic oral infection should be done to determine whether the relationship between periodontal disease and preterm is causal or simply associative.

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