

Analysis of CRP Level in Serum of Preeclamptic Women with Periodontal Disease

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Abstract:

Objective:

Preeclampsia is the major cause for fetomaternal complication affecting 5-10% of all pregnancy. Periodontitis has also been associated to be one among the causative factors for it. As these have adverse effects upon pregnant women, the present study was intended with an objective to determine the importance of an inflammatory marker C-Reactive protein level in pregnant women with periodontal disease and to correlate with the severity of periodontitis in preeclamptic pregnant women and also to determine the salivary pH, flow rate and specific gravity.

Methods:

The study included 60 preeclamptic pregnant women from whom 2 ml of venous blood was taken from the sample already collected for routine investigation. Informed consent were obtained from research participants. Estimation of CRP level was done from the collected samples using latex agglutination slide test. Simultaneously, periodontal examination was also done for these patients.

Results:

Among the 60 preeclamptic patients included in the survey, 53(88.3%) were noticed with mild periodontal disease and 7(11.7%) were noticed with moderate periodontal disease. The mean CRP level for patients with mild and moderate periodontal status was 1.155 ± 1.8 and 9.26 ± 9.4 respectively and the difference was statistically significant ($p = 0.001$).

Conclusion:

This showed that mean CRP level among patients with moderate periodontal diseases was higher than that of mild periodontal disease which indicates that CRP can be used as a marker of inflammation.

Key words: C-reactive protein, inflammation, inflammatory markers, periodontitis, preeclampsia.

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I. Introduction

Infections and inflammation is gradually increasing globally and has become a pandemic burden. Inflammation, especially, is known to play a vital role in the pathogenesis of various systemic and oral diseases in individuals and is associated and influenced with each other.¹

Among these, oral infections have been implicated and associated as causative agents in variety of systemic illness that includes atherosclerotic cardiovascular diseases and cerebrovascular ischemia. There are numerous oral infections such as dental caries, periapical infections, periodontal infections etc. among which, the microorganisms in periodontal diseases expose the host to microbial challenge, bacterial antigens and virulence factors leading to persistent inflammation that influence certain systemic diseases.

Studies have also shown that relationship exists between periodontitis and pregnant women, who, with poor oral health might be at higher risk of developing pregnancy complications such as preterm delivery and

low birth weight.² Also recent studies have shown the existence of relationship between higher prevalence of periodontitis and periodontal destruction in the preeclamptic women.

The prevailing disease burden awareness, proper diagnosis, identification of etiological and risk factors, frequent monitoring of disease activity and treatment are essential. Apart from routine clinical signs and symptoms, various laboratory investigations are also frequently used which may include the markers of inflammation such as CRP.³ CRP, is an acute phase protein and a direct measure of inflammation which is found to throw an immediate intensified response and sharply declining reflecting the exact inflammatory status, thus making it a better analyte.³

Periodontitis is one of the most common oral infections which directly or indirectly influences the systemic disease and it is of utmost importance to know about the effects it causes upon pregnant women, since, during pregnancy there is greater inflammatory response and very less importance is shown towards maintenance of proper oral health care. Various studies show that most women during pregnancy are affected by hypertension and proteinuria which results in the condition of preeclampsia also.

Preeclampsia is a common hypertensive disorder of pregnancy affecting 5 -10% of pregnancies and contributing significantly to maternal and perinatal morbidity and mortality. Despite the impact of this condition, efforts at understanding the etiological factors and measures designed to prevent preeclampsia is still disappointing.⁴

As obstetrics care for preeclamptic patients does not involve dental examination in most of the Indian hospitals and also there are limited studies relating to periodontitis in preeclamptic women of this region, the study is aimed to determine the CRP levels in pregnant preeclamptic women with periodontal disease, To correlate CRP level with severity of periodontitis in preeclamptic pregnant women and also to determine the salivary pH, flow rate and specific gravity.

II. Methods

This research work was approved by the Institutional Review Board (IRB ref no: IGIDSIRB2016NDP23PGCNOPM &01.12.16) and Institutional Ethical Committee, Indira Gandhi Institute of Dental Sciences (Ref no IGIDSIEC2016NDP23PGCNOPM & 16.12.16).The present Cross sectional study was conducted for the period of one and half a years (January 2017 to July 2018) to analyze the CRP level in serum of Preeclamptic women with periodontal disease at a private institution, Puducherry. Sample collection was carried out in the Department of Gynaecology and laboratory works were undertaken at the Department of Microbiology & Biochemistry.

Study subjects included 60 preeclamptic pregnant women with periodontal disease who attended Obstetrics and Gynaecology OPD. Inclusion criteria included Cases of Preeclampsia identified by proteinuria (presence of proteinuria \geq 0.3g in a 24 hr sample), Hypertension(\geq 140/90mmHg) assessed twice using standardized mercury Sphygmomanometer, Pregnant preeclamptic patients with periodontal disease (periodontitis with \geq 4mm of probing pocket depth).

Exclusion criteria included Pregnant women with cardiovascular or renal disease, Patients with other complications related to pregnancy and any major illness.

Two ml of venous blood was taken from the sample already collected from the patients (60 nos) for routine investigation. Patients were asked to refrain eating, drinking, 1hour before sample collection, allowed to rinse mouth using distilled water and then to relax for 5minutes. Patients were asked to lean their head forward over the container to allow the unstimulated whole saliva drain into the container for 5 minutes Collected sample was then transported to the Clinical chemistry laboratory, immediately for processing. Processing was carefully monitored by a trained Biochemist. The collected serum samples were delivered into EDTA TUBE, centrifuged in REMI table top centrifuge for 10 minutes at 3200 rpm and the serum separated by centrifugation was collected and stored at -20 °C. The Salivary samples collected were subjected to analysis. Salivary pH was calculated using pH meter, specific gravity by the mass / volume and flow rate of saliva by the quantity collected in 5 minutes. CRP level was estimated from the collected samples using latex agglutination slide test. Results were obtained, subjected to statistical analysis and values were tabulated in international units appropriately.

III. Results

The present study determined the CRP level in the serum of preeclamptic women with periodontal disease, correlated the CRP level with severity of periodontitis and also determined the salivary pH, flow rate and specific gravity in preeclamptic women.

All the available data were tabulated. Using independent t test it was found that there was no significant difference in the distribution of age, height, weight and gestational age in preeclamptic women with periodontal disease (**TABLE 1**).

Analyzing the parameters such as salivary pH, flow rate and specific gravity, it was found that there was no significant difference existed between the salivary pH, flow rate and specific gravity in preeclamptic women with periodontal disease (**TABLE 2**).

Among the 60 preeclamptic patients included in the survey, 53(88.3%) were noticed with mild periodontal disease and 7(11.7%) were noticed with moderate periodontal disease based on Russel's periodontal index (**TABLE 3**).

The mean CRP level for patients with mild and moderate periodontal status was 1.155 ± 1.8 and 9.26 ± 9.4 respectively and the difference was statistically significant ($p= 0.001$). This showed that the mean CRP level among patients with moderate periodontal diseases was higher than that of mild periodontal disease (**TABLE 4**).

IV. Discussion

Relationship exists between periodontitis and variety of systemic diseases, among which, the pregnant women with poor oral health are likely to be at higher risk of developing pregnancy complications such as preterm delivery and low birth weight^{1,2} Also recent studies have shown the existence of relationship between higher prevalence of periodontal destruction and periodontitis in the preeclamptic women. Therefore, it is essential to emphasize the contribution of periodontal pathogens in the oral diseases as periodontal disease has been presenting a major problem. Various organisms such as *A.actinomycetemcomitans*, *P.gingivalis*, *T.forsythensis*, *T.denticola*, *A.genus* has been found to be present in higher levels with periodontal disease and these microorganisms have been found to directly or indirectly influence the systemic conditions, one such condition being pregnancy.¹⁻⁵ *F.nucleatum* has been found to cause adverse outcomes in pregnancy associated with periodontal infection whose route of infection has been through the mother's bloodstream to placenta from the oral cavity⁵⁻⁹

During pregnancy, it has been hypothesized that in women with periodontal disease translocation of oral bacteria occurs to the circulation of both mother and the foetus resulting in inflammation of the placenta or oxidative stress¹⁰. In pregnancy, there is greater inflammatory response that maintains an equilibrium between the mother and the foetal allograft and is found to be induced by delivery^{11,12}. The inflammatory response activated is characterized by increase in the expression of activation markers on granulocytes and monocytes with production of cytokines and inflammatory proteins. This has been detected by the inflammatory markers and hepatic parameters.¹³

Intra uterine infections have been reported to contribute to 40-50% of all preterm births. Systemic maternal infections have been reported to lead to increased inflammatory cytokine levels which in turn stimulates the prostaglandin production thereby inducing uterine contractions and cervical ripening causing preterm parturition. High levels of interleukin-6 and interleukin-8 in serum are noticed during symptoms of preterm labour and associated with preterm birth. Measurement of circulating inflammatory markers have been suggested to be an alternate method for detecting women at high risk of preterm delivery.¹² Thus the prevailing disease burden, awareness, proper diagnosis, identification of etiological and risk factors, frequent monitoring of disease activity and treatment are essential to overcome the complications. Thereby various markers may be available to measure the inflammatory status among which one such is the C-reactive protein (CRP), which is considered as the hallmark for measuring inflammation.¹³

CRP, is an acute phase protein and direct measure of inflammation whose serum level is noticed to increase and later remain constant during the process of infection and inflammation. It throws an immediate intensified response and is sharply declining reflecting the exact inflammatory status, which makes it a better analyte for follow up studies.^{14,15} CRP is present in healthy individuals in less than 10 mg/l during infection or inflammation and is found to increase during the initial 6-8 hrs and reach peak levels of 350-400mg/l after a period of 48hrs with a half-life of approximately 19hrs.

Preeclampsia is a common hypertensive disorder of pregnancy affecting 5-10% of pregnancies and contributes significantly to maternal and perinatal morbidity and mortality.¹⁶ Despite the impact of this condition, efforts at understanding the etiological factors and measures designed to prevent preeclampsia is still disappointing. Does inflammation during pregnancy really result in preeclampsia with preterm birth? Obstetrics care for preeclampsia does not involve dental examination in most of the Indian hospitals and there are limited studies relating to periodontitis preeclamptic women in this region. Also since CRP measurement is relatively quick, noninvasive, and risk free, it can be used as a diagnostic test in evaluation and categorization of the risk levels and also in the anticipation of the morbidity of both the mother and fetus. Therefore, the aim of the present study was to determine the CRP level in preeclamptic pregnant women with periodontal disease, to correlate CRP level with severity of periodontitis in preeclamptic pregnant women and to determine salivary pH, flow rate, specific gravity in preeclamptic pregnant women with periodontitis.

Based on our first objective as to determine the CRP level in preeclamptic pregnant women with periodontal disease, the mean CRP for the sixty preeclamptic women was 2.10 ± 4.32 with a minimum of zero mg/dL and maximum of 19.2 mg/dL (Table -2).

On comparing the CRP level with periodontal disease (53 cases of moderate periodontitis and 7 cases of mild periodontitis), the mean CRP level for patients with mild and moderate periodontitis was 1.155 ± 1.8 and 9.26 ± 9.4 respectively and the difference was statistically significant ($p= 0.001$). This showed that mean CRP level among patients with moderate periodontitis was higher than that of mild periodontitis (Table- 4).

The reason for the increase in CRP in moderate periodontitis could be due to the periodontal pathogens that not only provoke local inflammation and tissue destruction, but may also involve in systemic increase of inflammatory and immune response. Another reason probably could be the activation of cascade of inflammatory cytokines by monocytes and other cells in the periodontal tissues.^{17,18} Inflammatory mediators especially IL-1 and IL-6 and tumour necrosis factor alpha are released under conditions of periodontitis thereby stimulating hepatocytes to produce CRP, although this controversy regarding the hypothesis of association between periodontitis and CRP still remains questionable. Another factor states that anaerobic gram negative bacterial species is found to be increased in dental plaque during pregnancy. This gram negative bacteria produces bioactive molecules such as LPS (lipopolysaccharides) which activates the macrophages and few other cells to synthesize IL-1 and IL-1 β , TNF- α , IL-6 and PGE2 which results in increase of CRP. The importance of this note is that these factors escape into the general circulation, cross the placental barrier resulting in elevation of CRP thereby resulting in preterm birth.^{11,12,19} Based on this association between the influence of periodontitis and elevated CRP level, preterm birth could be anticipated.

Evidence states that high blood pressure could influence the level of CRP. Since high blood pressure is one of the components of preeclampsia, elevated CRP level could be a finding in preeclampsia, which could serve as an important aid in anticipating preterm birth which is a complication of preeclampsia. Yet another concept says that the mother's psychological stress has got a significant role in stimulating pituitary-hypothalamus-adrenal axis and thereby release of cortisol hormone into the systemic circulation. Through this route, the hormone enters into the saliva, causing a reduction in the inflammatory cells inhibiting the immune response thereby causing inflammation of the periodontium.²⁰

Our study was concurrent with other studies which supported the association between CRP and periodontitis. Pitiphat et al. in their prospective cohort study showed that periodontitis increases the CRP level in pregnancy and he also suggested that the Nonsurgical periodontal therapy decreased the incidence of preterm birth and the levels of inflammatory mediators such as CRP.^{21,22} Zaima Ali et al., in his study determined the correlation of C-reactive protein levels in third trimester of pregnancy and found that, there was significant high levels of CRP when compared to that of normotensive women.²³ Ertas et al., in his study stated that elevated CRP level is an useful parameter in the severity of preeclampsia. Moreover, in his study, he measured the CRP using elisa kit and reported that there is a higher level of CRP in severe preeclamptic when compared to that of normal control and mild diseases.²⁴ In contrast, some studies of the same kind did not find significant role of CRP in pregnancies complicated by preeclampsia as compared to normotensive pregnant women, probably the reason could be attributed to the difference in sample size, timing of sample collection and CRP detection technique.²⁵⁻²⁷ Sharma A. et al., (2009), in their cohort study demonstrated that elevated CRP levels were noticed in pregnant women with periodontal disease when compared to healthy controls. It was also suggested that periodontal therapy before the 28 weeks of gestation caused significant decrease in levels of CRP. CRP, through amplification of the inflammatory response in complement activation, tissue damage and inducing inflammatory cytokines in monocytes was found to be associated with periodontal disease and adverse pregnancy outcomes.²⁸ Kumru S. et al, in his study in correlation of maternal serum in C-reactive protein levels with biochemical and clinical parameters in preeclampsia emphasized that preeclampsia increases the risk of intrauterine growth restriction and low birth weight. It was also found that placenta of both preeclamptic and IUGR pregnancies are characterized by a high apoptosis rate. This high apoptotic index may stimulate the release of CRP. Therefore CRP has got the function to serve as a scavenger for chromatin released from apoptotic cells, thereby promoting phagocytosis also. Thus it has been suggested that serum level of CRP may be related to placental functions in women, both healthy pregnant and with preeclampsia.²⁹ Maguire PA. et al. (2015), in their study showed that CRP concentration was higher in the first and eighteenth week of pregnancy when compared to the non pregnant adults.³⁰ Contrary to the above, there are studies that do not support the association of CRP with periodontitis and pregnancy. Lee CC. et al. 2012, in their study showed that CRP level was only reliable in distinguishing bacteremia from non bacterial infection.³¹ Touma L. et al. 2012, in their study have stated that periodontal disease was not associated with pregnancy and hence could not be considered as an independent risk factor for preterm labours.³²

The mean salivary pH for the preeclamptic women was found to be 6.75 ± 0.9 with a minimum of 5.2 pH units and maximum of 9.10 pH units. The mean Salivary flow rate for the preeclamptic women was

1.61±0.5 with a minimum of 0.70 ml/min and a maximum of 2.80 mL/min. The mean salivary specific gravity for the preeclamptic women was 1.003± 0.002 with a minimum of 1.0 g/ml and maximum of 1.01 g/ml.

There are studies showing that the salivary pH and flow rate is reduced in pregnancy. There are also studies which show increase in the salivary flow and decrease in the salivary pH of pregnant women. The reason for increase in salivary flow could be attributed to the increase in secretion of estrogen and progesterone while the decrease in the pH may be due to the decreased plasma HCO₃-ion concentration and increase in the α -amylase concentration. Evidence shows that the normal salivary pH is 6.2-7.4, salivary specific gravity is 1.0023 and the salivary flow rate is 0.3- 0.4 ml/min and an average of 4-5 ml/min during eating etc.

Based upon the results of our study, which was conducted in preeclamptic women for analyzing the salivary pH, flow rate and specific gravity, the mean salivary pH for the preeclamptic women was 6.75±0.9 with a minimum of 5.2 pH units and maximum of 9.10 pH units. The mean Salivary flow rate for the preeclamptic women was 1.61±0.5 with a minimum of 0.70 ml/min and a maximum of 2.80 mL/min. The mean salivary specific gravity for the preeclamptic women was 1.003± 0.002 with a minimum of 1.0 g/ml and maximum of 1.01 g/ml. From these results, we found that there was no significance in relation to the salivary pH, flow rate and specific gravity.

Thereby, the importance of this study is to stress upon the fact that elevated CRP levels during the early stages of pregnancy when associated with periodontal disease can be used as a biomarker to predict the subsequent development of preeclampsia in pregnant women. In addition to this, the inflammatory response is also found to result in the premature rupture of foeto placental membrane thereby suggesting this to be an indicator in the identification of such consequences.

V. Conclusion

From the results of this study, it has been inferred that there is a relationship of increase in the CRP level in preeclamptic women which would be a factor necessitating the importance to be identified and confined. Also, it denotes that CRP could definitely be used as a marker in this condition. It was also found that no direct relation existed between CRP that was influenced by the salivary pH, flow rate and specific gravity in preeclamptic women. Hence, longitudinal and interferential studies may be essential to establish further relationship between the association of these in preeclamptic women. Therefore the potential relationship between periodontal disease and CRP could be considered to be a significant factor for reducing the rate of preeclampsia and also for creating awareness during the public oral health programs in order to bring down the risk rate of other birth complications.

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TABULAR COLUMN

Table 1: Shows Background characteristics of preeclamptic women with periodontal disease

Variable	Mean ± SD	Minimum	Maximum	95% confidence Interval	
				Lower	Upper
Age	26.87 ± 4.21	18	37	25.78	27.95
Height	154.30 ± 5.13	143	168	152.97	155.63
Weight	70.29 ± 9.20	45.0	99.0	67.91	72.67
Gestational age	35.86 ± 3.75	20	40	34.88	36.82

The mean Age of the preeclamptic women was 26.87±4.21 with a minimum of 18 years and a maximum of 37 years. The mean height of the preeclamptic women was 154.30±5.13 with a minimum of 143 cms and a maximum of 168 cms. The mean weight of preeclamptic women was 70.29±9.20 with a minimum of 45.0 kgs and maximum of 99.0kgs. The mean Gestational age of the preeclamptic women was 35.86±3.75 with a minimum of 20 weeks and maximum of 40 weeks.

Table 2: Descriptive data for salivary pH, salivary flow rate, salivary specific gravity and CRP levels.

Variable	Mean ± SD	Minimum	Maximum	95% confidence Interval	
				Lower	Upper
Salivary pH	6.75 ± 0.92	5.20	9.10	6.51	6.99
Salivary Flow Rate	1.613 ±0.5	0.70	2.80	1.485	1.741
Salivary specific gravity	1.003 ±0.002	1.00	1.01	1.003	1.004
CRP	2.100 ± 4.32	0.0	19.2	0.984	3.216

The mean salivary pH for the preeclamptic women was 6.75±0.9 with a minimum of 5.2pH units and maximum of 9.10 pH units. The mean Salivary flow rate for the preeclamptic women was 1.61±0.5 with a minimum of 0.70 ml/min and a maximum of 2.80ml/min. The mean salivary specific gravity for the preeclamptic women was 1.003± 0.002 with a minimum of 1.0 g/ml and maximum of 1.01g/ml. The mean CRP for the preeclamptic women was 2.10 ± 4.32 with a minimum of zero mg/dL and maximum of 19.2mg/dL.

Table 3: Frequency distribution of periodontal disease

	Frequency	Percent
Mild	53	88.3
Moderate	7	11.7
Total	60	100.0

Among the 60 preeclamptic patients included in the survey, 53(88.3%) were noticed with mild periodontal disease and 7(11.7%) were noticed with moderate periodontal disease based on Russel’s periodontal index.

Table 4: Comparison of CRP level, salivary pH, flow rate and specific gravity between mild and moderate cases with periodontal disease Group statistics

	Periodontal status	N	Mean ± standard deviation	t	df	P
CRP	Mild	53	1.155±1.7605	5.82	58	0.001
	Moderate	7	9.257 ± 9.441			
Salivary pH	Mild	53	6.786 ± 0.956	1.18	10.3	0.264
	Moderate	7	6.471 ± 0.615			
Salivary flow rate	Mild	53	1.567 ± 0.502	1.99	58	0.050
	Moderate	7	1.957 ± 0.276			
Salivary specific gravity	Mild	53	1.003 ± 0.002	0.44	58	0.659
	Moderate	7	1.003 ± 0.002			

The mean CRP level for patients with mild and moderate periodontal status was 1.155 ± 1.8 and 9.26 ± 9.4 respectively and the difference was statistically significant ($p= 0.001$). It shows that mean CRP level among patients with moderate periodontal diseases was higher than that of mild periodontal disease.

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