Evaluation of the role of salivary C- reactive protein (CRP) as a diagnostic marker in hypertensive disorders of pregnancy and its correlation with periodontal status

Sumera Parveen*, Vivek Kumar Sharma*, Dalia Rafat**, Afshan Bey*, Moinuddin***, Rashid Jamal Khan$

*Department of Periodontics and Community Dentistry,Dr. Ziauddin Ahmad Dental College and Hospital (DRZADC), Aligarh Muslim University (AMU), Aligarh, Uttar Pradesh (U.P.), India.

**Department of Obstetrics and Gynecology, Jawaharlal Nehru Medical College and Hospital (JNMCH) , Aligarh Muslim University (AMU), Aligarh, Uttar Pradesh (U.P.), India.

***Department of Biochemistry, Jawaharlal Nehru Medical College and Hospital (JNMCH) , Aligarh Muslim University (AMU), Aligarh, Uttar Pradesh (U.P.), India.

$ Department of Medical Health & Family Welfare, Govt. of Uttar Pradesh, India.

Corresponding Author: Dr. Vivek Kumar Sharma, Professor and Chairman at Department of Periodontics and Community Dentistry,Dr. Ziauddin Ahmad Dental College and Hospital (DRZADC), Aligarh Muslim University (AMU), Aligarh, Uttar Pradesh (U.P.), India.

Mob:9058130053, Email:yeahlifehai@live.in

ABSTRACT

Background: There is limited information on the role of salivary C- reactive protein as a non invasive diagnostic marker in hypertensive disorders of pregnancy and its correlation with periodontal status. The study was aimed at evaluating and comparing the salivary CRP levels and clinical parameters in pregnant women with hypertensive disorders of pregnancy and in normotensive pregnant women.

Methods: One hundred pregnant women were randomly selected and divided into 2 groups: Group 1, pregnant women with hypertensive disorders of pregnancy ; Group 2, pregnant

women with normal blood pressure. Clinical periodontal parameters were recorded and biochemical assessment of salivary CRP levels were assessed and compared between both the groups.

Results: The outcome revealed higher mean salivary CRP levels and clinical parameters in pregnant women with hypertensive disorders of pregnancy compared with normotensive pregnant women. Also there was a significant correlation of salivary CRP with periodontal parameters.

Conclusion: Findings of our study provide support for the hypothesis that periodontal disease might increase the risk for hypertensive disorders of pregnancy, where salivary CRP might be a plausible mediator of association between both the diseases and thus could be used as a non invasive diagnostic tool in early detection and prevention of hypertensive disorders of pregnancy.

Keywords: C reactive protein, hypertensive disorders of pregnancy, periodontal disease.

INTRODUCTION

Hypertensive pregnancy disorders are a prominent cause of maternal and foetal morbidity and mortality, affecting 10% of all pregnancies.[1] These include Preeclampsia- eclampsia, Chronic Hypertension (CH), Chronic hypertension with superimposed preeclampsia and Gestational Hypertension (GH).[2] Among different types of hypertensive pregnancy disorders, preeclampsia (PE) a life threatening condition for both the mother and the foetus[3], is a pregnancy-specific disorder, whose impact have become one of the primary cause of maternal and foetal morbidity and mortality around the world, accounting for roughly 40% of all preterm births before 35 weeks of pregnancy. Although considerable amount of resources have been dedicated in prevention and treatment of different hypertensive disorders of pregnancy, it's still tough to anticipate, and thus tough to cure. Although the pathogenesis of

PE is uncertain, endothelial dysfunction of the maternal vascular system and infection are thought to play a role in the disease's clinical manifestations.[4-9]

Early treatment of urinary and vaginal infections can reduce the risk of pre-eclampsia[10], implying that infection may play a role in the pathophysiology of pre-eclampsia. And periodontal disease one of the most prevalent chronic oral infection causes persistent inflammation by exposing the host to microbial challenge, bacterial antigens, and virulence factors[11]. When it comes to hypertensive disorders during pregnancy, women with periodontal disease had a greater chance of developing PE than those who did not. Furthermore, the association has been linked to the severity of periodontal disease, when periodontitis is severe, the risk of PE is increased.[12] However, the biological mechanism underlying the link between periodontitis and PE is yet unknown.[13]

Periodontal disease is caused by oral bacteria, however periodontal destruction is predominantly regulated by the host's inflammatory response.[14,15] Daily episodes of bacteraemia or the spread of bacterial endotoxins originating from periodontal lesions have been suggested to activate the inflammatory response systemically[16,17] and cause the production of pro-inflammatory cytokines.[18] These cytokines further activate the inflammatory response, resulting in the up-regulation of interleukin-6 (IL-6), tumour necrosis factor (TNF-alpha), and C-reactive protein (CRP)[17,19-21], as well as generate a chronic low-grade systemic effect. Studies have shown that long-term exposure to periodontal pathogen generate a host response that may result in systemic maternal and placental pro-inflammatory endothelial activation and dysfunction, which is a major risk factor for vascular diseases like pre-eclampsia[22], one of the hypertensive disorders of pregnancy.

C-reactive protein (CRP) is an acute phase reactant produced by liver in response to the pro-inflammatory cytokines IL-6 and TNF. It is a marker of ongoing inflammation and tissue damage. CRP can be utilised as an early indicator of low-grade inflammation and can also aid

in the early detection of pathophysiological processes in pregnancy.[23] Therefore, CRP might be a plausible mediator of association between periodontitis and unfavourable pregnancy outcomes like preeclampsia and other hypertensive disorders of pregnancy.[24]

In this respect, periodontal disease in pregnant women may be another factor that influences the development of hypertensive disorders as a result of an increase in pro-inflammatory molecules both locally and systemically. Attempts are undertaken on a regular basis to have screening tests so that prompt preventive prophylactic interventions can be tried to avert unfavourable pregnancy outcomes.

Moreover, saliva is referred to as a "mirror of the body." Because of its origin, serum-like composition, and connections with other organs, salivary analysis has become an important tool for monitoring general health and disease. It contains biochemicals derived locally and systemically that has a high diagnostic value and could be utilised to diagnose several oral and systemic conditions. And, when compared to serum, salivary biomarkers have a number of advantages, including being a simple and non-invasive technique of collection, being quick and easy to collect, being cost effective, and so being an ideal substitute for serum.[25,26]

Therefore, measurement of salivary inflammatory markers may provide an alternative method of detecting women at risk for developing Hypertensive disorders of pregnancy.

So, the aim of present study is to evaluate the role of salivary C- reactive protein (CRP) as a diagnostic marker in hypertensive disorders of pregnancy and to correlate it with periodontal status.

AIMS & OBJECTIVES

1. To assess the level of salivary CRP in hypertensive disorders of pregnancy and in pregnant women with normal blood pressure.
2. To assess the periodontal status in hypertensive disorders of pregnancy and in pregnant women with normal blood pressure.

3. To correlate salivary CRP level with periodontal status

MATERIAL AND METHODS

STUDY DESIGN

The present study was designed as cross sectional observational study. The study was done by the Department of Periodontics and Community Dentistry, Dr. Z.A. Dental College & Hospital, AMU, Aligarh in collaboration with the Department of Obstetrics & Gynaecology and the Department of Biochemistry Jawaharlal Nehru Medical College and Hospital (JNMCH), Faculty of Medicine, AMU, Aligarh, India. The protocol was approved by Institutional Ethics Committee (IEC) of the institute with letter no. dated 16.10.20. The study was conducted in accordance with the ethical standards outlined in the declaration of Helsinki[27] (1975) as revised in 2013.

STUDY POPULATION

Subjects for this study were randomly selected from the Out-patient Department of Obstetrics & Gynaecology of the Jawaharlal Nehru Medical College and Hospital (JNMCH), AMU, using convenient sampling method. After the strict inclusion and exclusion criteria applied, a total of 100 patients were selected for the study without any bias on the basis of cast, religion or socioeconomic status and were asked to sign the informed consent form. They were further divided into two groups:

Group I: Pregnant women with hypertensive disorders of pregnancy

Group II: Pregnant women with normal blood pressure

SUBJECT SELECTION

Adequate sample of patients visiting the Department of Gynaecology & Obstetrics JNMC, AMU, Aligarh were randomly selected on the following basis- pregnant women in 3rd trimester of gestation of age group >18 and < 35years belonging to Indian ethnicity. Pregnant women with history of renal diseases**,** diabetes mellitus**,** cardiovascular diseases**,**

symptomatic infectious diseases**,** premature rupture of membranes, clinical chorioamnionitis, Invitro fertilisation were excluded.

Clinical Periodontal Measurements

Clinical periodontal measurements assessed included : Gingival bleeding index (Ainamo and Bay 1975)[28] and CPI Index (Community Periodontal Index) : by Jukka Ainamo[29], David Barmes, George Beagrie ,Terry Cutress, Jean Martin, and Jennifer Sardo –Infirri for joint working committee of the World Health Organisation (WHO) and Federation Dentaire Internationale (FDI) in 1982.

Saliva Sampling

The procedure as described by **Navazesh [30] et al** was followed for proper saliva collection – Patients were explained about the procedure and seated comfortably on a chair in an upright position and asked to rinse gently with water to remove any suspended impurities in the mouth. They were asked to relax for 5 minutes and thereafter 2 ml of unstimulated whole expectorated saliva was collected into 2ml saliva collecting tubes. Collected samples were labeled and stored at -20ºC in deep freezer.

Biomarker analysis

Salivary C-Reactive Protein (CRP) levels were determined for each subject utilizing commercially available human CRP ELISA kit provided by ELK Biotechnology (Cat : ELK1040).

Examination for hypertensive disorders of pregnancy

Examination for hypertensive disorders of pregnancy i.e. Gestational hypertension, chronic hypertension, chronic hypertension with superimposed preeclampsia, preeclampsia-eclampsia was performed by Gynaecologist in D/O Obstetrics & Gynaecology, JNMC, AMU, Aligarh according to ACOG Guidelines[31].

Statistical analysis

The normality of the data was tested using the Shapiro Wilk (SW) test. A p value less than 0.05 was considered to be statistically significant. The data was entered in a Microsoft excel spreadsheet and all the statistical analysis were performed with the help of SPSS (Statistical Package for Social Sciences) Version 23.0

RESULTS

Socio-Demography: The mean age, gestational age and body weight was compared between Group 1 and Group 2 using the unpaired t-test. Age, gestational age, educational level, socio-economic status and body weight were similar between the two groups. The mean age of participants in Group 1 and Group 2 were 28.14±2.77 and 27.40±2.56 years respectively, which was statistically insignificant (p=0.169). The percentage of smokers was comparable between both the groups (6% in group 1 and 4% in group 2), and did not affected the outcome of our study.

Salivary CRP levels (mg/l)

The mean CRP (mg/l) was compared between **Group 1** (1.65±1.24) and **Group 2** (0.55±0.70) using the unpaired t-test. The mean CRP (mg/l) was significantly more among Group 1 compared to Group 2 ($p<0.001$)

Periodontal status: Mean score of GBI ($p<0.001$), CPI ($p<0.001$), CPI-LOA ($p<0.003$), were significantly higher in group 1 compared to group 2.

A significant positive correlation of salivary CRP with periodontal parameters and blood pressure was noted. The results of our regression analysis (ΔR^2= 0.68 for SBP, 0.192 for DBP) for mediation of CRP between periodontal parameters and blood pressure in Hypertensive pregnant women showed that periodontal parameters have direct as well as indirect effect on Blood pressure, whereas CRP has strong indirect effect on blood pressure.

DISCUSSION

The present study was conducted to evaluate the role of salivary C-reactive protein (CRP) in hypertensive disorders of pregnancy and its correlation with periodontal status. Salivary C-reactive protein and periodontal parameters were assessed in hypertensive disorders of pregnancy and compared with normotensive pregnant women. The mean concentration of both salivary CRP levels and periodontal parameters were significantly higher in Group1 compared to Group 2. There was a significant correlation between salivary CRP and periodontal parameters. And interestingly the results of regression analysis (ΔR^2= 0.68 for SBP, 0.192 for DBP) also showed the same. The findings of our study and those from previous studies provide support for the hypothesis that periodontal disease might increase the risk for hypertensive disorders of pregnancy, where salivary CRP might be a plausible mediator of association between both the diseases and thus could be used as a non invasive diagnostic tool in early detection and prevention of hypertensive disorders of pregnancy. However , since periodontal parameters have direct as well as indirect effect on blood pressure as the results of mediation analysis found(ΔR^2= 0.68 for SBP, 0.192 for DBP) , hence some other mechanism could also be involved through which periodontal disease can cause hypertensive disorders of pregnancy.

There is accumulating evidence to show a link between periodontal disease in pregnant women

and the risk of unfavourable pregnancy outcomes such preterm birth[32], low birth weight[11],an dpre-eclampsia1[33],[21,34-36] and this link is reinforced by the discovery that periodontal disease activates the immune inflammatory response via a local increase in pro-

inflammatory mediators like interleukin-1b, prostaglandin E2, IL-6, and TNF-a. Periodontal disease is linked to an increase in C-reactive protein, which appears to be linked to the severity of the illness.[20] Furthermore; this molecule may play a role in the link between

periodontal disease and other systemic illnesses. Preeclampsia and periodontal disease have been linked epidemiologically in several studies.[12,37] The underlying process, however, is unknown, and the link between the two disorders has to be investigated further.

Boggess et al.[12] found that the presence of periodontal illness was related with a two-fold increase in the incidence of pre-eclampsia compared to women without periodontal infection in a longitudinal analysis of over 1000 women. In a research by Chaparro et al[38], patients with periodontitis who later had PE had higher plasma levels of CRP and a local increase in IL-6 in the GCF during early pregnancy. In this regard, periodontal disease in pregnant women may play a role in the development of hypertensive diseases by causing an increase in pro-inflammatory molecules both locally and systemically. In this respect, periodontal disease in pregnant women may be another factor that influences the development of hypertensive disorders as a result of an increase in pro-inflammatory molecules both locally and systemically.

To the best of our knowledge, we didn't find any previous studies which used CPI index, in context to role of CRP in hypertensive disorders of pregnancy and its correlation with periodontal status. Thus, we cannot compare our findings much. But since probing depth and loss of attachment are a part of CPI index, so we tried to compare our findings to a certain extent. In the study done by ***Contreras* et al[37]** reported that Probing Depth (PD) and Clinical Attachment Loss (CAL) was more in pre-eclamptic group compared to control, which was statistically significant, which is in agreement with our study. Studies of **Cota LOM et al[39], Canacki et al[22]**, **Swati Pralhad et al[40]** also found that pre-eclamptic women had worse periodontal health than normotensive women, which further supports are study.

In contrary to our study, Khader et al[41] and **Lohsoonthorn et al[42]** found no statistical differences between pre-eclamptic cases and normotensive controls with regard to mean periodontal probing depth, mean clinical attachment loss and bleeding on probing. However,

studies of Khader et al[41] and Lohsoonthorn et al[42] et al did not support our study, but the possible explanation for this could be due to: Firstly, as they have assessed only clinical parameters which are subjected to examiner based error or bias that can change upon examination by different examiner on contrary to a biomarker which is a more valuable predictor of hypertensive disorders of pregnancy. Moreover, the timing of the assessment of these parameters in relation to hypertensive disorders was inconsistent. Secondly, heterogeneity in type of population might also be attributable to differences in findings.

Salivary CRP status

The mean CRP (mg/L) was significantly more among Group 1 compared to **Group 2** ($p<0.01$), which is in accordance with the study done by **Chapparo et al.[38]** There are studies which had evaluated the role of CRP levels in hypertensive pregnant women but they had not included and or correlated with periodontal disease. Studies of **Rashmi RR et al[43] K. Kameswaramma[44] and Raio Luigi et al[45]** are few of them which also found significantly raised CRP levels in hypertensive group, which is in agreement with our study.

Understanding the pathogenesis of both diseases may aid us to recognize pregnant women who are at risk, so as to minimise the risk during treatment.[46] Furthermore, interventional studies examining the effect of periodontal treatment on systemic markers revealed a decrease in serum CRP levels and an improvement in endothelial function over the course of 6 months[47]. Hence, periodontal treatment could be started at least 6 months before conception or at the start of pregnancy to minimise poor birth outcomes like PE and other hypertensive disorders of pregnancy.

To the best of our knowledge , studies of **Herrera et al,[48] Chapparo et al[38] and N Chitra et al[49]** are the only few researches that have looked into the role of CRP in the link between periodontal disease and PE. But our study is unique in the sense that, till date it is the only study that involves role of salivary CRP in hypertensive disorders of pregnancy and its

correlation with periodontal status. The present study was conducted on unstimulated saliva rather than serum/ plasma which has been used in above mentioned studies, as the detection of salivary biomarker has several advantages compared to serum, including the simple and non- invasive method of collection, rapid and easy to collect, cost effective and no special equipment and expertise required. Several studies have examined the relationship between salivary and serum CRP. One study reported the corresponding sensitivity and specificity for salivary CRP to discriminate a serum CRP is found to be 0.64 and 0.94, respectively. And statistically significant correlation has been found between serum and salivary CRP ($r = 0.62$, $p < 0.001$)[50]. Hence salivary CRP as a non invasive tool is more advantageous compared to serum.

Our study's strength was that we had the stringent inclusion and exclusion criteria and study population was derived from the same ethnic background. Also along with the clinical periodontal parameters (GBI, CPI, CPI-LOA) we have reinforced our findings with the biochemical analysis of salivary CRP which is considered a more valuable predictor of hypertensive disorders of pregnancy and is not subjected to any examiner based error or bias contrary to clinical parameters that can change upon examination by different examiner.

But still careful interpretation of our data should be employed, as the etiology of both periodontal disease and preeclampsia is likely multifactorial. Also some important limitations should be kept in mind when evaluating the results of present study: the study had a small sample size, cross sectional study design, moreover we did not categorise different hypertensive disorders of pregnancy into different groups and assessment accordingly. And also we did not categorise periodontal status based on severity. Therefore, we feel that prospective longitudinal studies with a larger sample size are required to evaluate the role of salivary CRP as a possible mediator of association between hypertensive disorders of

pregnancy and periodontal disease and also their predictive value for the development of hypertensive disorders of pregnancy.

REFERENCES

1. Wagner SJ, Barac S, Garovic VD. Hypertensive pregnancy disorders: current concepts. The Journal of Clinical Hypertension. 2007 Jul;9(7):560-6.
2. American College of Obstetricians and Gynecologists. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' task force on hypertension in pregnancy. Obstetrics and gynecology. 2013 Nov;122(5):1122-31.
3. Carreiras M, Montagnani S, Layrisse Z. Preeclampsia: a multifactorial disease resulting from the interaction of the feto-maternal HLA genotype and HCMV infection. American Journal of Reproductive Immunology. 2002 Sep;48(3):176-83.
4. Faas MM, Schuiling GA, Baller JF, Visscher CA, Bakker WW. A new animal model for human preeclampsia: ultralow-dose endotoxin infusion in pregnant rats. Am J Obstet Gynecol 1994;171:158– 164.
5. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science 2005;308:1592–1594.
6. Roberts JM, Gammill HS. Preeclampsia: recent insights. Hypertension, 2005;46: 1243–1249.
7. Roberts JM. Endothelial dysfunction in preeclampsia. Semin Reprod Endocrinol 1998;16:5–15.
8. Chavarria ME, Lara-Gonzalez L, Garcia-Paleta Y, Vital-Reyes VS, Reyes A. Adhesion molecules changes at 20 gestation weeks in pregnancies complicated by preeclampsia. Eur J Obstet Gynecol Reprod Biol 2008;137:157–164.
9. Veenstra van Nieuwenhoven AL, Moes H, Heineman MJ, Santema J, Faas MM. Cytokine production by monocytes, NK cells, and lymphocytes is different in

preeclamptic patients as compared with normal pregnant women. Hypertens Pregnancy 2008;27: 207–224

10. Herrera JA, Chaudhuri G, Lopez-Jaramillo P. Is infection a major risk factor for preeclampsia? Med Hypotheses 2001; 57:393–397.
11. Li X, Kolltveit KM, Tronstad L, Olsen I. Systemic diseases caused by oral infection. Clin Microbiol Rev 2000; 13:547–558
12. Boggess KA, Lieff S, Murtha AP, Moss K, Beck J, Offenbacher S. Maternal periodontal disease is associated with an increased risk for preeclampsia. Obstet Gynecol 2003;101:227–231.
13. Kunnen A, Van Doormaal JJ, Abbas F, Aarnoudse JG, van Pampus MG, Faas MM. Periodontal disease and preeclampsia: a systematic review. J Clin Periodontol 2010;37:1075–1087.
14. Offenbacher S, Boggess KA, Murtha AP et al. Progressive periodontal disease and risk of very preterm delivery. Obstet Gynecol 2006;107:29–36.
15. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet 2005; 366:1809–1820.
16. Geerts SO, Nys M, De MP et al. Systemic release of endotoxins induced by gentle mastication: association with periodontitis severity. J Periodontol 2002;73: 73–78.
17. Loos BG, Craandijk J, Hoek FJ, Wertheim-van Dillen PM, van der Velden U. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. J Periodontol 2000;71:1528–1534.
18. Scannapieco FA. Periodontal inflammation: from gingivitis to systemic disease? Compend Contin Educ Dent 2004;25: 16–25.
19. Moutsopoulos NM, Madianos PN. Low grade inflammation in chronic infectious diseases: paradigm of periodontal infections. Ann N Y Acad Sci 2006;1088:251– 264.

20. Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. J Clin Periodontol 2008;35:277–290.

21. Nakajima T, Honda T, Domon H et al. Periodontitis associated upregulation of systemic inflammatory mediator level may increase the risk of coronary heart disease. J Periodontal Res 2009;45:116–122.

22. Canakci V, Canakci CF, Yildirim A, Ingec M, Eltas A, Erturk A. Periodontal disease increases the risk of severe pre-eclampsia among pregnant women. Journal of clinical periodontology. 2007 Aug;34(8):639-45.

23. Du Clos TW. The interaction of C- reactive protein and serum amyloid P component with nuclear antigens. Mol Biol Rep. 1996;23(3-4):253-60.

24. Cermak J, Key NS, Bach RR, Balla J, Jacob HS, Vercellotti GM. C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. Blood 1993;82:513-20.

25. Alwan AH, Taher MG, Getta HA, Hussain AA. Estimation of the level of Salivary Interleukin 6 (IL-6) and its' correlation with the clinical parameters in patients with periodontal diseases. IOSR J Dent Med Sci. 2015 Sep;14(9):82.

26. Lima DP, Diniz DG, Moimaz SA, Sumida DH, Okamoto AC. Saliva: reflection of the body. International Journal of Infectious Diseases. 2010 Mar 1;14(3):e184-8.

27. World Medical Association. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Bulletin of the World Health Organization. 2001;79(4):373.

28. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. International dental journal. 1975 Dec;25(4):229.

29. Ainamo J, Barmes D, Beagrie G, Cutress T, Martin J, Sardo-Infirri J. Development of the World Health Organization (WHO) community periodontal index of treatment needs (CPITN). Int Dent J. 1982 Sep;32(3):281-91. PMID: 6958657

30. Navazesh M. Methods for collecting saliva. Annals of the New York Academy of Sciences. 1993 Sep;694(1):72-7.

31. Govindaraju P, Venugopal S, Shivakumar MA et al. Maternal periodontal disease and preterm birth: A case-control study. Journal of Indian Society of Periodontology. 2015;19(5):512-515.

32. Marcaccini AM, Meschiari CA, Sorgi CA, Saraiva MC, de Souza AM, Faccioli LH, Tanus-Santos JE, Novaes Jr AB, Gerlach RF. Circulating interleukin-6 and high-sensitivity C-reactive protein decrease after periodontal therapy in otherwise healthy subjects. Journal of periodontology. 2009 Apr;80(4):594-602.

33. Lopez NJ, Smith PC, Gutierrez J. Higher risk of preterm birth and low birth weight in women with periodontal disease. J Dent Res 2002; 81: 58–63

34. Van Dyke TE, Sima C. Understanding resolution of inflammation in periodontal diseases: is chronic inflammatory periodontitis a failure to resolve?. Periodontology 2000. 2020 Feb;82(1):205-13.

35. Oettinger-Barak O, Barak S, Ohel G et al. Severe pregnancy complication (preeclampsia) is associated with greater periodontal destruction. J Periodontol 2005;76: 134–137.

36. Riche´ EL, Boggess KA, Lieff S et al. Periodontal disease increases the risk of preterm delivery among preeclamptic women. Ann Periodontol 2002;7:95–101.

37. Contreras A, Herrera JA, Soto JE, Arce RM, Jaramillo A, Botero JE. Periodontitis is associated with preeclampsia in pregnant women. J Periodontol. 2006 Feb;77(2):182–8.

38. Chaparro A, Blanlot C, Ramírez V, Sanz A, Quintero A, Inostroza C, et al. Porphyromonas gingivalis, Treponema denticola and toll-like receptor 2 are associated with hypertensive disorders in placental tissue: a case-control study. J Periodontal Res. 2013 Dec;48(6):802–9.

39. Cota LOM, Guimarães AN, Costa JE, Lorentz TCM, Costa FO. Association between maternal periodontitis and an increased risk of preeclampsia. J Periodontol. 2006 Dec;77(12):2063–9.

40. Pralhad S, Thomas B, Kushtagi P. Periodontal disease and pregnancy hypertension: a clinical correlation. J Periodontol. 2013 Aug;84(8):1118–25.

41. Khader YS, Jibreal M, Al-Omiri M, Amarin Z. Lack of association between periodontal parameters and preeclampsia. J Periodontol 2006;77:1681-1687.

42. Lohsoonthorn V, Kungsadalpipob K, Chanchareonsook P, et al. Maternal periodontal disease and risk of preeclampsia: A case-control study. Am J Hypertens 2009; 22:457-463.

43. Rashmi Ranjan Rout, Meenakshi Mahalik .Comparison of C-reactive proteins level in gestational hypertension and in normal pregnancy in 2nd and 3rd trimester and its correlation with maternal and foetal outcome. *Int J Reprod Contracept Obstet Gynecol. 2019 Jun;8(6):2541-2548*

44. Kameswaramma K. Estimation of C-reactive protein, magnesium and uric acid levels in preeclampsia patients in comparison with normal pregnant women. Sch. J. App. Med. Sci. 2014;2(2B):628-32.

45. Raio L, Bersinger NA, Malek A, Schneider H, Messerli FH, Hürter H, et al. Ultra-high sensitive C-reactive protein during normal pregnancy and in preeclampsia: a pilot study. J Hypertens. 2019 May;37(5):1012–7.

46. Michalowicz BS, Hodges JS, DiAngelis AJ et al. Treatment of periodontal disease and the risk of preterm birth. N Engl J Med 2006;355:1885–1894.

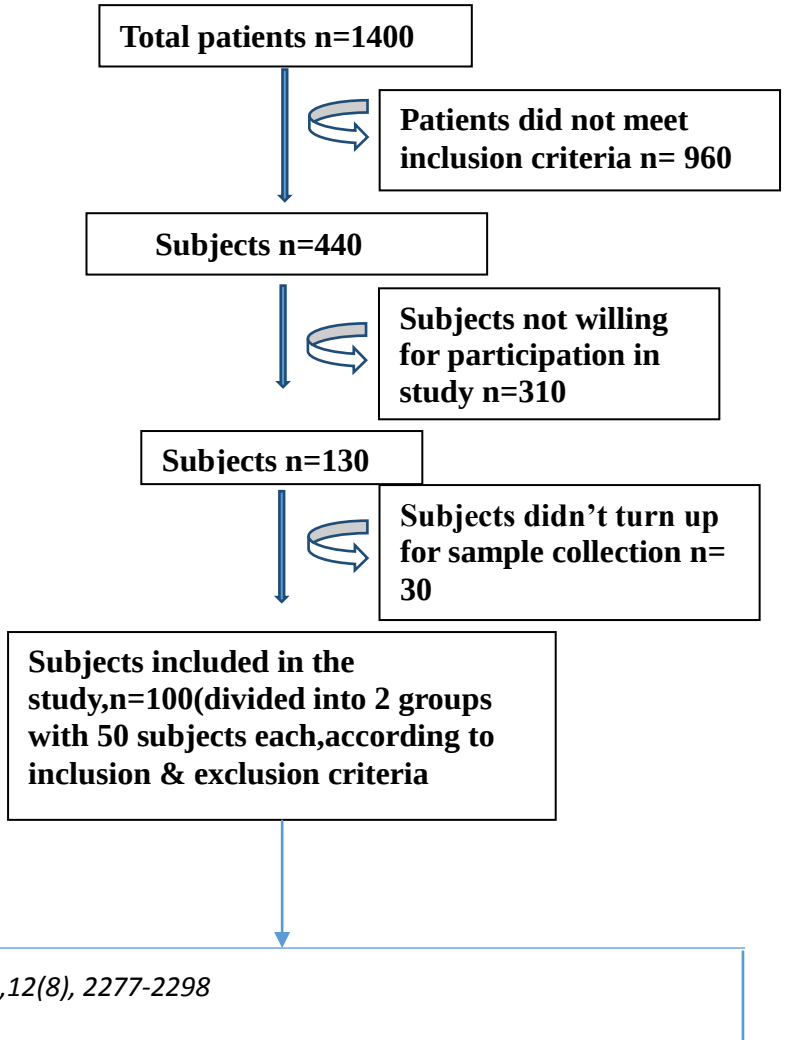
47. Tonetti MS, D'Aiuto F, Nibali L et al. Treatment of periodontitis and endothelial function. N Engl J Med 2007;356:911 –920

48. Herrera JA, Parra B, Herrera E, Botero JE, Arce RM, Contreras A, et al. Periodontal disease severity is related to high levels of C-reactive protein in pre-eclampsia. J Hypertens. 2007 Jul;25(7):1459–64.

49. Chitra N, Santhadevy A, Premlal KR, Pallavee P, Sathish Babu M, Suganya R. Analysis of CRP level in serum of preeclamptic women with periodontal disease. IOSR J Dental Med Sci. 2019;18(5):83-9.

50. Iyengar A, Paulus JK, Gerlanc DJ, Maron JL. Detection and potential utility of C-reactive protein in saliva of neonates. Frontiers in pediatrics. 2014 Nov 21;2:131.

FLOW CHART 1: SUBJECT ALLOCATION AND STUDY DESIGN



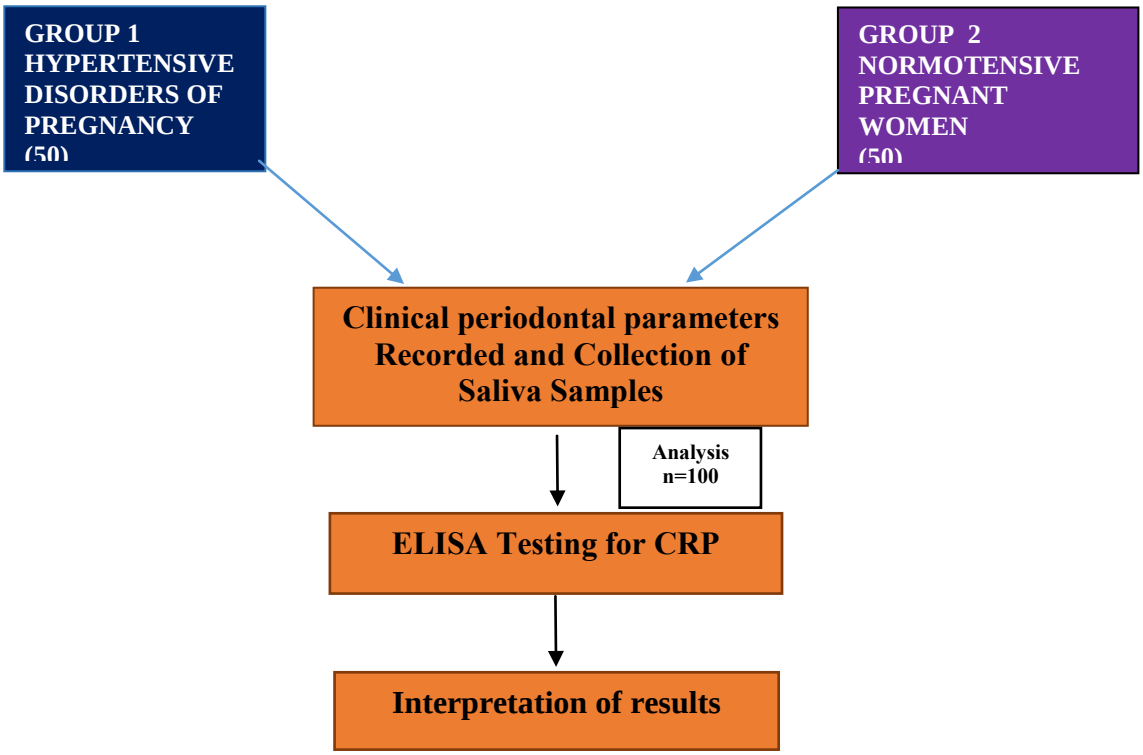
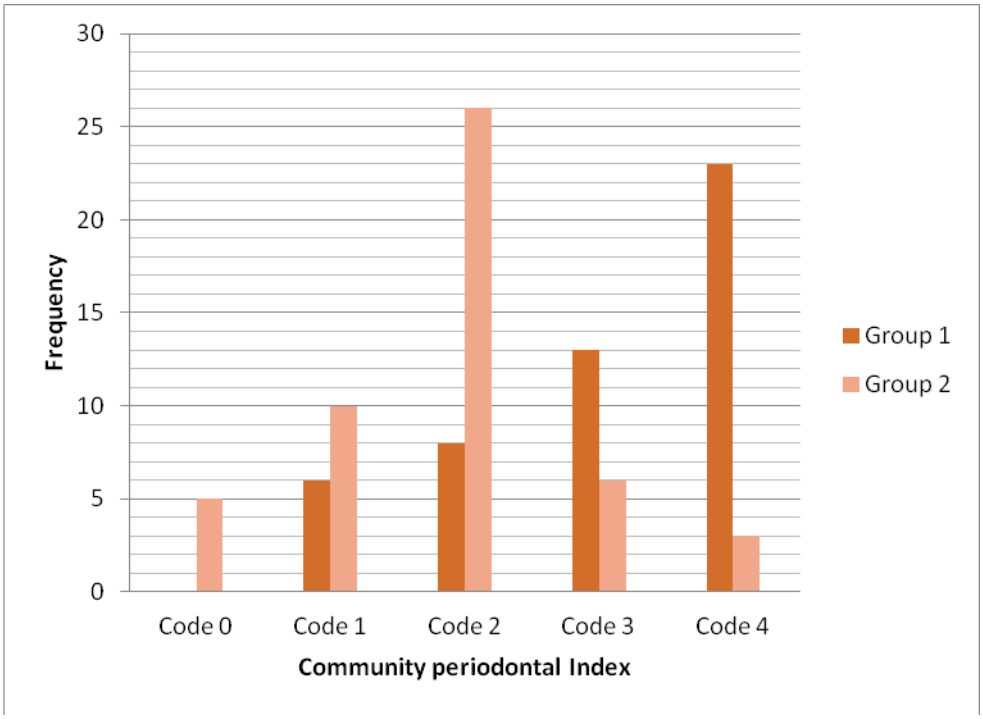


TABLE 1: Socio-Demographic Profile of Participants under Study

Socio-demographics	Mean ±SD		p-value
	Group 1	Group 2	
1. Age	28.14±2.77	27.40±2.56	0.169
2. Gestational Age(weeks)	34±2.05	32±2.77	0.980
3. Illiterate(percentage)	60	65	0.075
4. Socioeconomic status (percentage)	Lower(20) Middle(65)	Lower(22) Middle(68)	0.296

	Upper(15)	Upper(10)	
5. Smoking (percentage)	6	4	0.860
6. Body weight (Kg)	78.12±6.56	78.86±4.36	0.946

Unpaired t- test **#Non- significant difference**

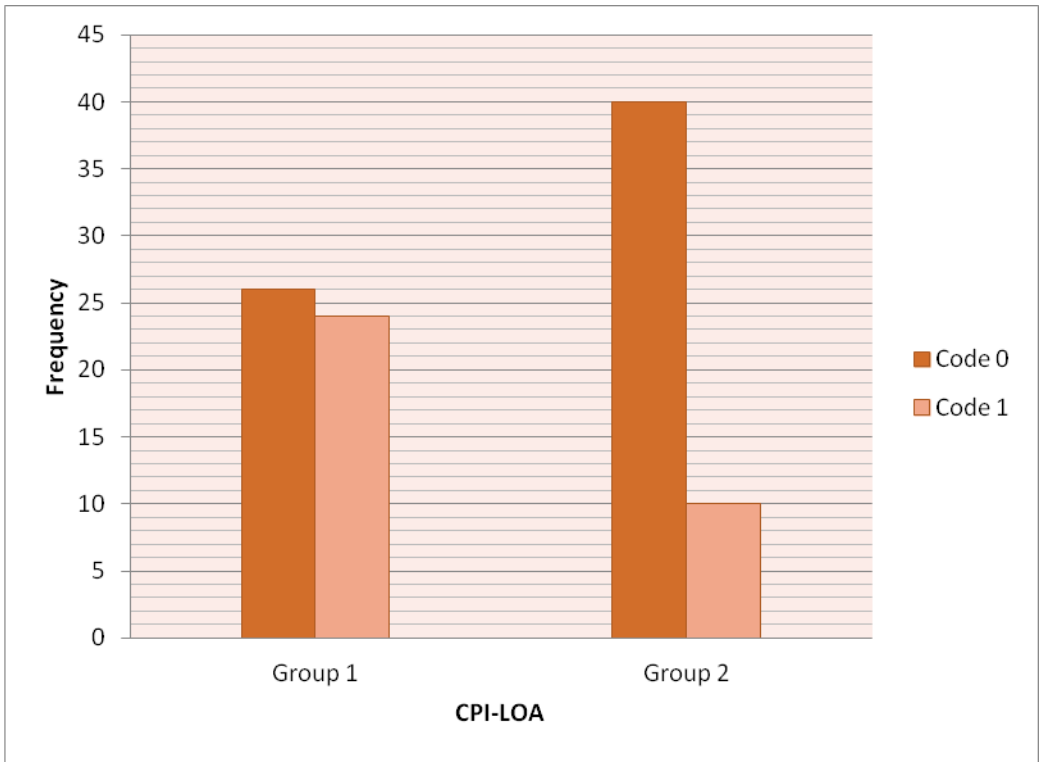


Graph 1: Bar Graph for Frequency Distribution of CPI in Two Groups

****significant at .01 level of significance**

Note: χ^2 is chi-square test and df is degree of freedom; percentages are out of 100%;

CPI is Community Periodontal Index.



Graph 2: Bar Graph for Frequency Distribution of CPI-LOA in Two Groups

**significant at .01 level of significance

Note: CPI-LOA is Loss of Attachment.

Table 2: Correlation Coefficient between Different Variables under Study and their Significant Level in Both the Groups

Variables	Group 1 n=50		Group 2 n=50		Overall	
	r	p	r	p	r	P
CRP-CPI	.388**	.005	.507**	.000	.563**	.001
CRP-GBI	.416**	.003	.653**	.000	.642**	.001
CRP-CPILOA	.339*	.016	.880**	.000	.561**	.001
CRP-Systolic	.434**	.002	.398**	.004	.577**	.001
CRP-Diastolic	.574**	.001	.397**	.004	.596**	.001

***significant at .05 level of significance**

****significant at .01 level of significance**

Note: r is the value of correlation.

Table 3 : Regression Analysis for Mediation of CRP between Periodontal Parameters and Systolic and Diastolic Blood Pressure in Hypertensive Group (Group 1)

Variable	**B**	**95% CI**	**SEB**	**B**	**R^2**	**ΔR^2**
Step 1 Constant Periodontal parameters	136.16** .220**	(129.35,142.966) (0.101,0.338)	3.385 .059	.474**	.225	.225**
Step 2 Constant Periodontal parameters CRP	136.71 .165 1.530	(130.11,143.30) (.039,.290) (.081,2.979)	3.277 .063 .720	.355** .286*	.293	.068*
B.Mediation of CRP between Periodontal Parameters and Diastolic Blood Pressure						
Variable	**B**	**95% CI**	**SEB**	**B**	**R^2**	**ΔR^2**
Step 1 Constant Periodontal	88.43**	(85.27,91.60)	1.574	.370**	.137	.137**

parameters	.076**	(0.020,0.131)	.027			
Step 2						
Constant	90.41**	(89.26,91.56)	.573			
Periodontal parameters	.076**	(0.020,0.131)	.027	.370**	.329	.192**
CRP	1.351**	(.791,1.910)	.278	.574**		

Note CI= Confidence interval
* p< .05 level of significance.
** p < .01 level of significance.