Original Article

Evaluation of Serum C-reactive Protein Levels in Subjects with Aggressive and Chronic Periodontitis in Comparison with Healthy Controls: A Clinico-biochemical Study

Vijayalakshmi Bolla, P Santha Kumari[1], Surendra Reddy Munnangi[2], D Sunil Kumar[1], Y Durgabai[1], Pradeep Koppolu[3]

Department of Periodontology, S.V.S. Institute of Dental Sciences, Mahabubnagar, Telangana, [1]Department of Periodontology, Government Dental College and Hospital, Hyderabad, [2]Department of Pedodontics and Preventive Dentistry, S.V.S. Institute of Dental Sciences, Mahabubnagar, Telangana, India, [3]Department of Preventive Dental Sciences, Al-Farabi Colleges, Riyadh, KSA

Abstract

Aim: Evaluation and comparison of serum C-reactive protein (CRP) levels in subjects with chronic and aggressive periodontitis. **Materials and Methods:** Based on the periodontal status, 45 subjects were selected and divided into three groups. Group I - subjects with clinically healthy periodontium, Group II - generalized aggressive periodontitis (GAP), and Group III - chronic periodontitis (CP). Blood samples were collected from subjects for measurement of CRP. Periodontal parameters include plaque index (PI), gingival index, bleeding index (BI), probing pocket depth (PPD), and clinical attachment loss (CAL) were assessed. CRP levels were assessed by means of a commercially available high sensitivity-CRP enzyme immunoassay kit. **Results:** CRP levels were increased in Group III (6.0671 ± 3.15639 mg/L) and Group II subjects (4.5453 ± 2.88116 mg/L) compared to the Group I (1.0180 ± 0.94069 mg/L). CRP levels showed a positive correlation with all clinical parameters in Group I subjects. BI (r = 0.073), PI (r = 0.120) showed a positive correlation with CRP level in Group II and a positive correlation was also seen for PI (r = 0.492), PPD (r = 0.340), CAL (r = 0.160), and CRP level in Group III subjects. **Conclusion:** The mean CRP levels were found to be greater in CP compared to GAP subjects, but there was no statistically significant difference.

Keywords: *Aggressive periodontitis, Chronic periodontitis, C-reactive protein*

Introduction

Several reports have suggested that longstanding nature of periodontal disease results in the development of atherosclerosis, cardiovascular disease (CVD), cardiovascular accident, preterm low birth weight infants,[1,2] and changes in the lipid profile.[3] Risk factors for atherosclerosis include many factors, which also include subgingival Gram-negative pathogens as their presence on the atherosclerotic heart valves.[4,5] They release cytokines, involved in the destruction of periodontal tissues,[6] initiation of systemic acute phase response, and release of acute phase reactants such as C-reactive protein (CRP), fibrinogen, and serum amyloid A.[7]

CRP is a pentameric molecule and has several properties which include antibacterial, upregulation of proinflammatory cytokine production, a decrease in the development of autoimmune diseases, stimulation of repair, regeneration of a variety of tissues, and foam cell formation in atheromas.[8,9] In healthy individuals, CRP levels are found in trace amounts, i.e., <0.3 mg/l. Subjects with concentrations more than 3 mg/l in serum CRP are considered to be at higher a risk for future cardiovascular diseases and events.[10-12] After controlling for established risk factors which contribute to the elevation of CRP levels, increased levels of CRP persisted among individuals with the extensive periodontal disease.[13,14]

Chronic periodontitis (CP) is slow, and Aggressive periodontitis is rapid in disease progression. The estimation of CRP levels in periodontitis subjects may give the indication of an underlying pathway in the association between periodontal disease, CVD,[15] and impaired blood glucose levels.[16]

Materials and Methods

Forty-five (25 male and 20 female) subjects aged between 25 and 50 years, with mean age of 28.9 ± 1.2 and those who have not received any antibiotic therapy and periodontal therapy in the last 3 months with a minimum number of 20 teeth in the

How to cite this article: Bolla V, Kumari PS, Munnangi SR, Kumar DS, Durgabai Y, Koppolu P. Evaluation of serum c-reactive protein levels in subjects with aggressive and chronic periodontitis in comparison with healthy controls: A clinico-biochemical study. Int J App Basic Med Res 2017;7:121-4.

Received: 05 June, 2016.
Accepted: 11 September, 2016.

Address for correspondence:
Dr. Vijayalakshmi Bolla, Department of Periodontology, S.V.S. Institute of Dental Sciences, Mahabubnagar - 509 002, Telangana, India.
E-mail: drmvijaya@gmail.com

Access this article online

Website: www.ijabmr.org

DOI: 10.4103/2229-516X.205814

Quick Response Code:

oral cavity, with probing depth of ≥5 mm and with clinical attachment loss (CAL) ≥2, with good systemic health were included for the study from Department of Periodontics, Government Dental College, Hyderabad, India after getting the ethical clearance from the institute. Subjects were excluded if they had history of known systemic diseases and presence of other chronic infections, smokers and alcoholics, pregnant or lactating females, treatment with any medication known to affect the serum CRP levels, such as antibiotics, nonsteroidal anti-inflammatory drugs, oral contraceptive pills, 3-hydroxy-3-methylglutaryl-coenzyme A inhibitors in the past 3 months.

Based on periodontal status, subjects were divided into three groups by computer generated randomization. Group I (subjects with healthy periodontium), Group II generalized aggressive periodontitis (GAP), and Group III chronic generalized periodontitis. At baseline, blood samples were collected from subjects for measurement of CR *P* value. Periodontal parameters recorded included, plaque index (PI),[17] gingival index (GI),[17] bleeding index (BI),[18] probing pocket depth (PPD) (distance from gingival margin to the base of the sulcus), CAL (distance from cementoenamel junction to the base of the pocket). For each subject, the periodontal disease status was evaluated at four sites per tooth by two examiners (Buccal, Distobuccal, Mesiobuccal, and lingual) measured using UNC 15 probe (Hu-Friedy's, USA).

Ten milliliter of blood sample was collected from the brachial vein from each of the subjects, by aseptic technique using a 10cc syringe, transferred to an appropriately labeled tube and centrifuged for 15 min at 3000 RPM separating the cells from the serum and the smear layer was removed carefully.

Separated serum was collected in eppendorf and stored in the deep freeze at - 20°C. Serum CRP levels were assessed by means of a commercially available high sensitivity-CRP Enzyme Immunoassay* (The EiAsy™ Way, Diagnostics Biochem, Canada Inc., Canada).

Mean values of each parameter were compared between the groups using one-way analysis of variance with *post hoc* test of least significance difference method. Relationships between the parameters were assessed by Pearson's correlation coefficient. Analysis of covariance was used for comparison of mean values between the groups to adjust the ages. The test values were considered statistically significant at $P < 0.05$.

Results

The mean age of the subjects in Groups I, II, and III were 31.13 ± 8.55 years, 29.27 ± 3.77 years, and 36.73 ± 7.38 years, respectively. This difference was found to be statistically not significant ($P = 0.27$). The mean PI scores in the Groups I, II, and III were 0.69 ± 0.17, 1.63 ± 0.57, and 1.71 ± 0.30, respectively. The mean BI scores in the Groups I, II, and III were 36.10 ± 15.97, 94.96 ± 10.48, and 93.10 ± 6.79, respectively. The mean GI scores in the Groups I, II, and III were 0.46 ± 0.21, 2.07 ± 0.52, and 2.1 ± 0.52, respectively. The mean CAL scores in the Groups I, II, and III were 1.30 ± 0.31 mm, 4.34 ± 0.64 mm, and 4.37 ± 0.75 mm, respectively [Table 1].

The mean CRP concentration in the Groups I, II, and III was 1.01 ± 0.94 mg/L, 4.54 ± 2.88 mg/L, and 6.06 ± 3.15 mg/L, respectively. Intergroup comparison showed statistically significant difference ($P = 0.012$) in the CRP level between Groups I and II, and between Groups II and III, and between Groups I and III [Figure 1].

A statistically significant difference was found in the PPD and CRP scores of Groups I and II, Groups I and III, and Groups II and III. CRP levels did not show a positive correlation with all clinical parameters in Group I subjects. BI ($r = 0.073$), PI ($r = 0.120$) showed a positive correlation with CRP level in group II and a positive correlation was also seen for PI ($r = 0.492$), PPD ($r = 0.340$), CAL ($r = 0.160$) and CRP level in Group III subjects. The difference in the mean scores of PI, BI, GI, and CAL Groups I and II, and Groups I and III was found to be statistically significant ($P < 0.05$), whereas the difference in the BI scores of Group II and III was not found to be statistically significant ($P = 0.25$) [Table 2].

Discussion

The present study showed an increase in serum CRP levels in CP and GAP subjects compared to the controls. This is in accordance with the results of earlier studies which have shown an elevation in CRP levels in periodontitis patients.[10,13,19,20]

In this study, more than 50% of subjects with CP and GAP demonstrated a mean CRP level >3 mg/L, the level of CRP concentration reported to be at risk for development of cardiovascular disease. The findings of the present study are in agreement with similar studies by Slade *et al*.[13] and Wu *et al*.[21] showed a significant correlation between periodontal status and prevalence of elevated CRP. However, these studies used the NHANES III database and CRP levels <3 mg/L were not measured in these studies.

Table 1: Comparison of periodontal parameters among the groups

	Mean±SD			*P**
	Group I	Group II	Group III	
PI	0.695±0.1704	1.637±0.574	1.711±0.306	0.004
GI	0.466±0.212	2.072±0.528	2.108±0.528	0.137
BI	36.109±15.971	94.961±10.480	93.673±6.796	0.25
PPD	2.046±0.555	5.598±0.834	4.424±0.410	0.017
CAL	1.301±0.311	4.346±0.642	4.376±0.759	0.010

*One-way analysis using *post hoc* test. CAL: Clinical attachment loss; PPD: Probing pocket depth; BI: Bleeding index; GI: Gingival index; PI: Plaque index; SD: Standard deviation

Fredriksson *et al*.,[22] Ebersole *et al*.,[23] Koppolu *et al*.[24] found higher serum CRP concentrations in periodontitis patients than in controls. While Ebersole *et al*. observed a relationship between CRP levels and the absence or presence, or severity of, adult periodontitis, it is not evident whether the study design controlled for the effects of smoking, a known factor for both serum markers concentration and periodontitis.[23] The later study reported about 9 mg/L, roughly one and half times that of the CRP concentrations of the present study. This difference could be caused by the investigation of more severe periodontitis cases in their study. Furthermore, their study did not adjust for potential confounders such as age, smoking, body mass index (BMI), and blood lipids in particular, since hyperlipidemia has been associated with periodontal disease.[25,26]

Very few studies have evaluated CRP levels in aggressive periodontitis subjects.[10,18,16] One study by Salzberg *et al*.[10] reported an increase in CRP levels in GAP subjects (3.72 mg/L) and the observations are similar to the findings of the present study (4.54 mg/L). In the present study, though the CRP levels were found to be elevated in aggressive periodontitis group of subjects, the mean CRP levels were found to be lower when compare to CP group. The reason for this difference in CRP levels between aggressive and CP groups is not exactly understood at this point of time, but could be attributable to the long standing nature and chronic course of the disease process in CP, thus exerting its systemic influence over a longer period compared to aggressive periodontitis which runs a shorter course.[27]

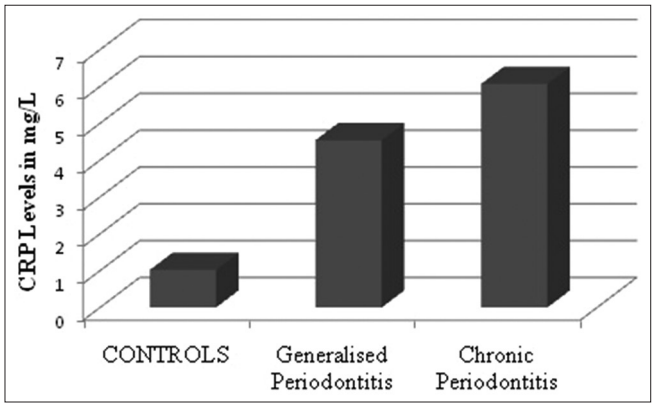

Figure 1: Comparison of mean C-reactive protein levels among the groups

Table 2: Correlation between C-reactive protein levels with parameters

Correlation co-efficient (*r*) of CRP	Group I		Group II		Group III	
	r	*P**	*r*	*P**	*r*	*P**
CRP versus age	−0.060	0.831	0.453	0.090	0.130	0.658
CRP versus PI	0.062	0.827	0.120	0.670	0.492	0.074
CRP versus BI	0.134	0.635	0.073	0.797	−0.044	0.881
CRP versus GI	0.061	0.942	−0.125	0.658	0.317	0.269
CRP versus PPD	0.081	0.775	−0.297	0.282	0.340	0.234
CRP versus CAL	0.348	0.204	−0.306	0.267	0.160	0.584

*Pearson correlation test. CAL: Clinical attachment loss; PPD: Probing pocket depth; BI: Bleeding index; GI: Gingival index; PI: Plaque index; CRP: C-reactive protein

In addition to chronic infections, CRP serum concentrations are reported to be positively correlated with age, smoking, BMI, or lipid parameters. Since the mean age among the groups differed significantly in the present study, with the lowest being in the control group followed by aggressive periodontitis group and CP group, age was included as a potential confounding parameter in covariance analysis. Such an analysis showed that the age did not influence the mean CRP levels of the study.[28]

In the present study, clinical parameters such as bleeding on probing (BOP) showed a positive correlation with CRP level in aggressive periodontitis group and a positive correlation was also seen for PPD, clinical attachment level, and CRP in CP group of subjects. This is in accordance with the earlier studies which reported that increased BOP, probing depth and attachment loss to be significantly associated with elevated CRP concentrations.[10,17,28]

Glurich *et al*.[28] found significantly elevated CRP levels in subjects with BOP and mean CAL ≥4 mm. Noack *et al*.[19] found a significant increase of serum CRP in subjects with 3 mm mean CAL. Slade *et al*.[13] reported a mean CR *P* value of 4.5 mg/L in subjects with ≥10% of siteS with PPD ≥4 mm. These findings indicate a dose – response relationship between the extent of periodontitis and CRP. The results of the present study corroborate the observations of the previous studies indicating that periodontal diseases are associated with elevated serum CRP levels. Elevation of CRP such as that seen in periodontal diseases may supplement systemic vascular inflammation, atheroma formation and add to the preexisting risk for cardiovascular sequelae. If periodontitis can lead to the elevation of CRP levels, then theoretically, periodontal therapy should help in reducing the systemic burden of inflammation. However, to know whether such benefit can really be translated in the long-term can only be assessed in future by well-controlled longitudinal clinical trials. Further studies should focus on the relationship between periodontitis, elevated CRP levels and the effect of periodontal therapy on serum CRP concentration. The results of the present study indicated an increase in serum CRP levels in subjects with GAP and CP compared to controls. The CRP levels in CP subjects were higher when compared to subjects with aggressive periodontitis, but this was not found to be statistically significant. However, the results of the present study cannot be used to determine the causality of the associations between periodontitis and CRP due to some limitations, one being the small sample size and the

other is that the study is only cross-sectional. Moreover, the subjects might have undiagnosed systemic factors and other undetected risk factors that could influence the CRP levels. It would be appropriate if large sample based, well controlled, longitudinal trials are performed to determine the relationship between periodontitis and elevated CRP levels and effect of periodontal therapy on serum CRP concentration.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Williams RC, Offenbacher S. Periodontal medicine: The emergence of a new branch of periodontology. Periodontol 2000 2000;23:9-12.
2. Radafshar G, Shad B, Mirfeizi M. Association between periodontal disease and elevated C-reactive rotein in acute myocardial infarction patients. J Dent Tehran Univ Med Sci 2006;3:126-34.
3. Flores MF, Montenegro MM, Furtado MV, Polanczyk CA, Rösing CK, Haas AN. Periodontal status affects C-reactive protein and lipids in patients with stable heart disease from a tertiary care cardiovascular clinic. J Periodontol 2014;85:545-53.
4. Ross R. Atherosclerosis – An inflammatory disease. N Engl J Med 1999;340:115-26.
5. Pejcic A, Kesic L, Milasin J. Association between periodontopathogens and CRP levels in patients with periodontitis in Serbia. J Dent Res Dent Clin Dent Prospects 2011;5:10-6.
6. Page RC. The role of inflammatory mediators in the pathogenesis of periodontal disease. J Periodontal Res 1991;26(3 Pt 2):230-42.
7. Senthil N, Vandana KL, Sangeeta D. A study to qualitatively evaluate the serum levels of C-reactive protein (CRP) in various inflammatory periodontal diseases. J Indian Soc Periodontol 2001;5:27-30.
8. Ebersole JL, Cappelli D. Acute-phase reactants in infections and inflammatory diseases. Periodontol 2000 2000;23:19-49.
9. Pepys MB, Hirschfield GM. C-reactive protein: A critical update. J Clin Invest 2003;111:1805-12.
10. Salzberg TN, Overstreet BT, Rogers JD, Califano JV, Best AM, Schenkein HA. C-reactive protein levels in patients with aggressive periodontitis. J Periodontol 2006;77:933-9.
11. D'Aiuto F, Ready D, Tonetti MS. Periodontal disease and C-reactive protein-associated cardiovascular risk. J Periodontal Res 2004;39:236-41.
12. Thakare KS, Deo V, Bhongade ML. Evaluation of the C-reactive protein serum levels in periodontitis patients with or without atherosclerosis. Indian J Dent Res 2010;21:326-9.
13. Slade GD, Offenbacher S, Beck JD, Heiss G, Pankow JS. Acute-phase inflammatory response to periodontal disease in the US population. J Dent Res 2000;79:49-57.
14. Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. J Clin Periodontol 2008;35:277-90.
15. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 2002;347:1557-65.
16. Susanto H, Nesse W, Dijkstra PU, Hoedemaker E, van Reenen YH, Agustina D, *et al.* Periodontal inflamed surface area and C-reactive protein as predictors of HbA1c: A study in Indonesia. Clin Oral Investig 2012;16:1237-42.
17. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963;21:533-51.
18. Turesky S, Gilmore ND, Glickman I. Reduced plaque formation by the chloromethyl analogue of Vitamin C. J Periodontol 1970;41:41-3.
19. Noack B, Genco RJ, Trevisan M, Grossi S, Zambon JJ, De Nardin E. Periodontal infections contribute to elevated systemic C-reactive protein level. J Periodontol 2001;72:1221-7.
20. Al-Shahat MA, Al-Samad AA, El-Aal IA. Aggressive and slowly-progressive periodontitis in patients with coronary artery diseases. Levels of C-reactive protein, fibrinogen, and IL-6. Cairo Dent J 2008;24:141-53.
21. Wu T, Trevisan M, Genco RJ, Falkner KL, Dorn JP, Sempos CT. Examination of the relation between periodontal health status and cardiovascular risk factors: Serum total and high density lipoprotein cholesterol, C-reactive protein, and plasma fibrinogen. Am J Epidemiol 2000;151:273-82.
22. Fredriksson MI, Figueredo CM, Gustafsson A, Bergström KG, Asman BE. Effect of periodontitis and smoking on blood leukocytes and acute-phase proteins. J Periodontol 1999;70:1355-60.
23. Ebersole JL, Machen RL, Steffen MJ, Willmann DE. Systemic acute-phase reactants, C-reactive protein and haptoglobin, in adult periodontitis. Clin Exp Immunol 1997;107:347-52.
24. Koppolu P, Durvasula S, Palaparthy R, Rao M, Sagar V, Reddy SK, *et al.* Estimate of CRP and TNF-alpha level before and after periodontal therapy in cardiovascular disease patients. Pan Afr Med J 2013;15:92.
25. Cutler CW, Shinedling EA, Nunn M, Jotwani R, Kim BO, Nares S, *et al.* Association between periodontitis and hyperlipidemia: Cause or effect? J Periodontol 1999;70:1429-34.
26. Lösche W, Karapetow F, Pohl A, Pohl C, Kocher T. Plasma lipid and blood glucose levels in patients with destructive periodontal disease. J Clin Periodontol 2000;27:537-41.
27. Kanaparthy A, Kanaparthy R, Niranjan N. Evaluation of serum C-reactive protein levels in subjects with aggressive and chronic periodontitis and comparison with healthy controls. Dent Res J (Isfahan) 2012;9:261-5.
28. Glurich I, Grossi S, Albini B, Ho A, Shah R, Zeid M, *et al.* Systemic inflammation in cardiovascular and periodontal disease: Comparative study. Clin Diagn Lab Immunol 2002;9:425-32.