ORIGINAL RESEARCH

Effect of periodontal treatment on serum levels of cardiovascular disease predictor c - reactive protein in patients with chronic periodontitis

Fajar Ummar, Manoj Raja, Mathew Thomas, Rekha P Thankachan, Sameer KM

Department of Periodontics, MES Dental College, Perintalmanna, Kerala and Department of Periodontics, Karpaga Vinayaga Institute of Dental Sciences, Chennai

Correspondence to: fajarummar@gmail.com

Abstract

Recent evidences indicate that C-reactive protein (CRP) and CRP inducing cytokines may be involved indirectly as well as directly in the development of atherosclerosis and hence can be validated as CVD predictor. It has been proposed that patients with periodontitis may have elevated circulating levels of these inflammatory markers like c-reactive protein and hence increase the risk for atherosclerosis. The aim of this present study was to determine whether the presence of chronic periodontitis and subsequent non surgical periodontal treatment could influence the serum levels of C-reactive protein (CRP).

In this study, a total of 60 patients were assessed for the estimation of serum C-reactive protein. The test group included 30 patients with chronic periodontitis and the control group included 30 periodontally healthy individuals. Serum blood samples were collected at baseline for both the groups and three months after therapy for the study group for the estimation of C-reactive protein levels by immunoturbidometric method. It was found that the mean CRP level of the patients with chronic generalized periodontitis was significantly higher than that of the healthy controls and significant reduction in these levels was observed after non surgical therapy. It was also found that there was no statistically significant difference between the post treatment (90^{th} day) CRP levels of chronic generalized periodontitis group and the healthy controls which indicate that the non surgical therapy brought down the CRP levels of chronic periodontitis patients to levels comparable with that of healthy controls.

Introduction

Periodontal diseases are chronic gram negative oral infections initiated in the gingiva and leading to alveolar bone destruction and gradual loss of tooth supporting connective tissues. Cumulatively these infections affect more than 70% of the general population.[1] Recent evidence associates periodontal disease with a higher risk for atherosclerotic plaque formation that could lead to myocardial infarction, ischemic stroke and peripheral arterial disease. Several pathological mechanisms have been proposed to explain this strong association, including the involvement of systemically elevated inflammatory mediators.

C-reactive protein is a well known acute phase reactant produced by the liver in response to diverse inflammatory stimuli including heat, trauma, infection and hypoxia. Their levels provide useful information for the diagnosis, monitoring and therapy of inflammatory process and associated disease.

C-reactive protein has been shown to play a role in the pathogenesis of atherosclerosis through different mechanisms including binding the phosphocholine of oxidized low density lipoproteins, upregulating the expression of adhesion molecules in

endothelial cells, increasing low density lipoprotein uptake into macrophages, inhibiting endothelial nitric oxide synthase expression in aortic endothelial cells, and increasing plasminogen activator inhibitor-1 expression and activity.[2]

Recent studies have demonstrated a correlation between periodontitis and elevated CRP levels. It was found that after controlling for established risk factors, increased levels of CRP persisted among individuals with extensive periodontal disease. In fact, among dentate individuals with extensive periodontal disease, an increase of approximately one third in mean CRP and a doubled prevalence of elevated CRP were found compared to periodontally healthy subjects. It was also observed that subjects with periodontal disease and cardiovascular disease demonstrated higher levels of CRP than subjects with cardiovascular disease and no periodontitis.[3] There was also a significant increase in adjusted mean levels of CRP in subjects with high attachment loss when compared to subjects with healthy periodontium.[3]

Given the results of various studies, if we can say that periodontal infection does contribute significantly to the systemic levels of CRP, then it would follow that periodontal treatment could result in its reduction also and hence decrease the risk for atherosclerosis. Previous studies have shown that periodontal treatment lead to the reduction of serum CRP levels.[4]

The present study was undertaken to examine the serum levels of CRP in periodontitis and the effect of periodontal treatment on the serum level of this inflammatory marker.

Materials and methods

A total number of 60 subjects with age range of 30-60 years were selected for this study. They were divided into two groups in which Group I: (Control group) consisted of thirty individuals who were free of periodontal disease and GROUP II (test group) consists of thirty individuals who were diagnosed with generalized mild to moderate chronic periodontitis, with 4-6 mm probing pocket depth along with radiographic evidence of bone loss. Patients under medications like antibiotics,

corticosteroids, anti-inflammatory drugs and oral contraceptive pills for the past 3 months, systemic diseases, who have undergone periodontal treatment in the last 6 months, smokers and pregnant woman and patients with less than 24 teeth were excluded from the study. The clinical parameters that were assessed at baseline (0 day) and on 90th day were probing pocket depth and clinical attachment level.

Study design

I. 0 day (baseline)

The subjects selected for the study (30 patients with chronic periodontitis and 30 healthy controls) were referred to the biochemistry laboratory of for collection of non fasting venous blood for the estimation of C-reactive protein.

On the same day, the test group patients underwent supragingival scaling with ultrasonic (piezoelectric) scalers. The patients were then recalled after one week for subgingival scaling and root planing which was done with the aid of gracey curettes (area specific). Oral hygiene instructions were given to the patients.

II. *90th day*

The patients were recalled on the 90th day after non surgical therapy (scaling and root planing). The clinical parameters that were assessed at baseline (probing pocket depth and clnical attachment level) were reassessed. The patients were then referred to the biochemistry laboratory for the collection of serum and estimation of CRP.

Estimation of C-reactive protein

5µl of the serum sample was taken and 450µl of reagent 1 and 50µl of reagent 2 were added to it and the absorbance was read from semi automated analyzer. Serum levels of CRP of each patient were quantified using Particle Enhanced Turbidometric Immunoassay (PETIA) technique. The lower and upper detection limits of the kit being 0.1 – 20mg/ml. All the samples were automatically quantified by the computer assisted semi automated biochemical analyzer.

Results

The mean CRP levels of test group were estimated as 3.317mg/l and 2.260mg/l at baseline and 90th day. The mean CRP level of control group at baseline was found to be 1.93mg/l.

The comparative analysis of clinical parameters as well the CRP levels were done using various statistical tools and their outcome is shown in the following tables and graphical representations.

Table 1: CRP levels on day 0 and day 90 in group 1 patients

TIME POINT	N	Mean CRP Level in mg/l	STD DEVIATION	STD ERROR MEAN
Day 0	30	3.317*	1.0732	.1959
Day 90	30	2.260	1.0516	.1920

- 'P' Value< 0.001 (Statistically significant

Table 2. CRP levels in patients of group 1 and group 2 on day 0

Group	Mean CRP level ±S.D	P value
Group I	3.317±1.07*	*< 0.001
Group II	1.93±0.94	

Table 3. CRP levels in patients of group 1 on day 90 and group 2 on day 0

Group	N	Mean CRP level	Std Deviation	STD Error Mean
GROUP I	30	2.260	1.0516*	.1920
GROUP II	30	1.933	.9349	.1707

- p= .209

Table 4. Probing pocket depth on day 0 and day 90 in patients of group 1

TIME POINT	MEAN PROBING POCKET DEPTH ±S.D	P value
DAY 0	4.383±.6660*	*.000
DAY 90	3.243±.6072	

Table 5. Clinical attachment level on day 0 and day 90 for patients of group 1

TIME POINT	MEAN CLINICAL ATTACHMENT LEVEL ±S.D	P value
Day 0	4.480±.7083*	*.000
Day 90	3.310±.6609	

Figure 1. CRP levels on day o and day 90 in patients of group 1

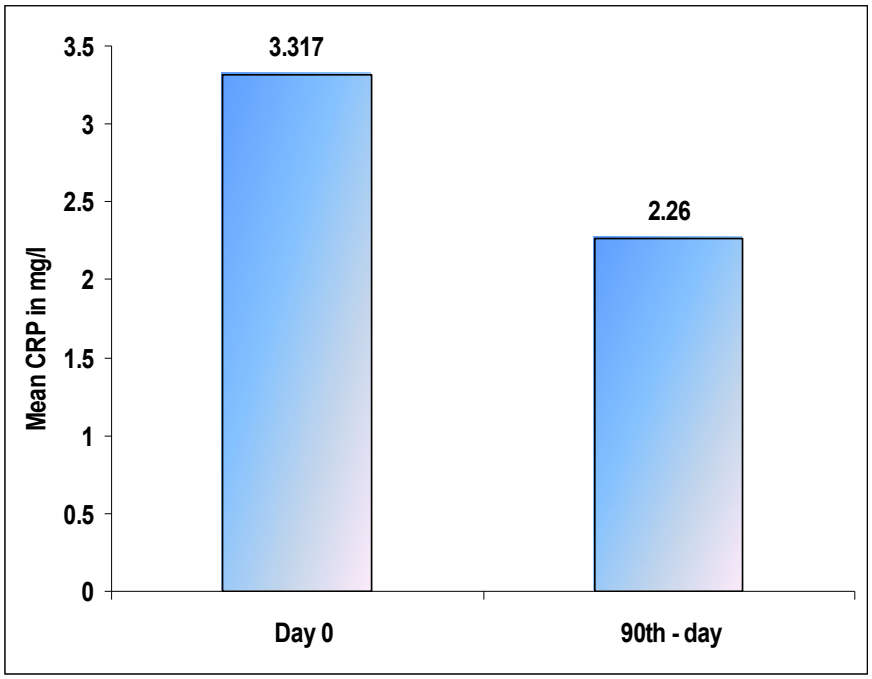


Figure 2. CRP levels in patients of group 1 and group 2 on day 0

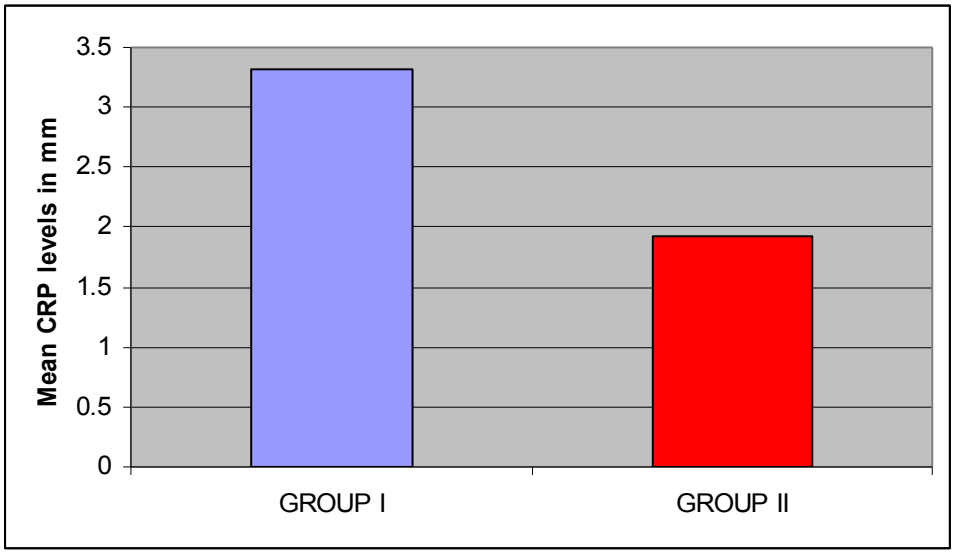
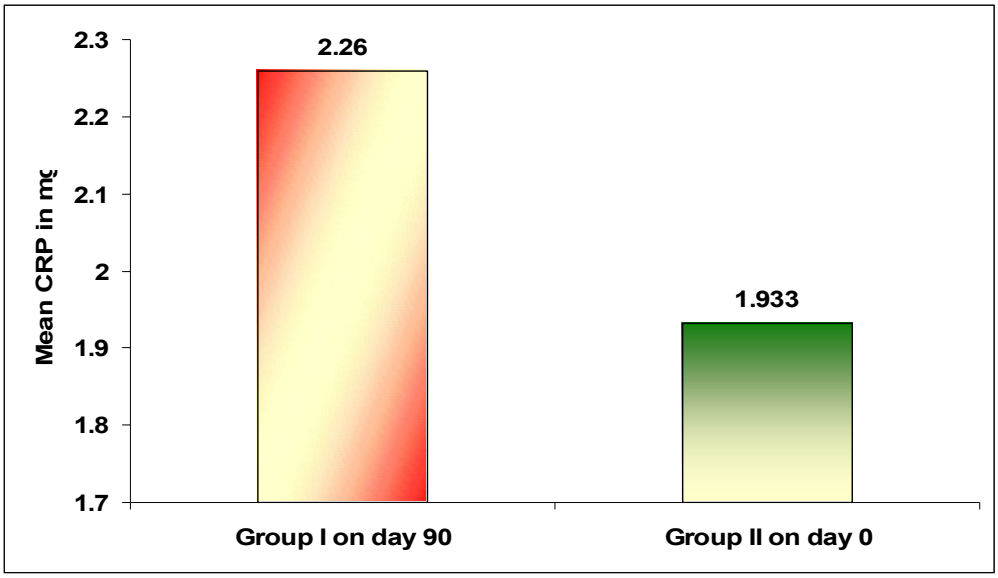


Figure 3. CRP levels in patients of group 1 on day 90 and group 2 on day 0



Discussion

After two decades of research, it has been established that an association exists between periodontal and cardiovascular disease. The pertinent question is however about the nature and relevance of this association and whether the infectious and inflammatory periodontal disease process contributes casually to heart attacks and strokes or whether it's a coincidental association. There are strong indications that the inflamed and ulcerated pocket epithelium forms an easy portal of entry for oral microorganisms. Also bacterial endotoxins (LPS) and all kinds of microbial agents can be disseminated throughout the body originating from the periodontal lesion. In addition to the conventional risk factors for atherosclerosis, such as hypertension, hyperlipidemia and diabetes, the role of inflammatory mediators is intrinsic to the concept of atherosclerosis as a chronic inflammatory process. The LPS and inflammatory cytokines that are present in periodontal diseases may also increase the expression of leukocyte adhesion molecule (ICAM) or vascular cell adhesion molecule (VCAM) by endothelial cells which in turn are associated with atheroma formation. Herzberg and colleagues in 1983[5], Herzberg and Mayer 1996[6] have proposed a direct effect of some of the bacteria found in dental plaque that enters the blood stream during a bacteraemic episode. The oral gram positive bacteria streptococcus sanguis and gram negative periodontal pathogen *Porphyromonas gingivalis* have been shown to induce platelet activation and aggregation through expression of collagen like platelet aggregation associated protein. The aggregated platelet may then play a role in atheroma formation and thrombosis.

Even though it has been affirmed that infection with periodontopathic bacteria can induce a strong inflammatory response, it has not been fully elucidated as to how much the local inflammatory response in the periodontium can influence the systemic levels of inflammatory mediators and acute phase proteins.. And also, if periodontal infection contributes significantly then it would follow that periodontal treatment could result in the resolution of systemic inflammation and hence decrease the risk for atherosclerosis.

So the present study was undertaken to evaluate C-reactive protein levels in chronic periodontitis patients and the effect of non surgical periodontal therapy on these levels. It was noted that serum CRP levels in subjects of group I tended to be higher at baseline (Day0) compared to that of group II. The mean CRP level of test group and control group on day 0 were 3.317±1.0732 mg/l and 1.933±0.9349mg/l respectively. The mean difference of CRP level between group I and group II was 1.587±0.335 which was statistically significant (P <0.001).

This is in accordance with study by Beck *et al*[7] where he observed statistically significant increase in CRP level in chronic periodontitis patients (3.48 ± 2.64 mg/l) compared to controls (1.2 ± 1.34 mg/l). Study by Loos BG *et al* [8] also had similar results.

The elevated level of CRP in chronic periodontitis group compared to the healthy individuals may be due to the systemically dispersed bacteria and LPS as well as cytokines from periodontal lesions which may stimulate hepatocytes in liver to produce CRP.[9] When the mean CRP level of group I on day 0 and day 90 were compared, there was statistically significant reduction. This data demonstrated that basic non surgical periodontal treatment in patients with moderate chronic periodontitis results in a statistically significant resolution of systemic inflammation. Our findings are in accordance with the results of studies done by Yashihiro Iwamoto *et al* [10], Honda YK *et al*[11] , Lalla E *et al* [12], Al Zahrani MS[13] and Farin Kiany Yazdi *et al.*[14]

CDC/AHA workshop has categorized the patients with CRP levels greater than 3mg/l as high risk group for cardiovascular disease (CVD). So according to the above criteria the group I subjects (CRP levels -3.317 mg/l) of our study fall under the borderline category of high risk group for CVD. It was also observed that CRP levels of this group reduced to a statistically significant level (2.260 mg/l) and thereby emphasizing the fact that the chances of risk for CVD can be progressively reduced with periodontal therapy. it has been suggested that CRP localizes with

complement in the human heart during myocardial infarction, CRP binds to diseased muscle tissue, fixes complement and hence triggers complement- mediated inflammation that contributes to atheroma formation.[15] American Heart Association advocates the use of CRP as a predictor for heart diseases. Hence the elevated level of CRP in chronic periodontitis and decrease in CRP after periodontal debridement strongly suggest that this may be a causative factor in genesis of CVD by periodontal infection. Further studies will be required to validate the usefulness of examining acute phase reactants in monitoring periodontal diseases.

Conclusion

The findings of our study supports the hypothesis that the rise in systemic inflammatory marker CRP observed in periodontitis patients can be reduced by periodontal treatment, thereby reducing the cardiovascular disease risk. Long term studies with a larger study population in cardiovascular disease patients having severe periodontitis will be required to further substantiate the association.

References

1. Albandar JM, Brunelle JA, Kingman A. Destructive periodontal disease in adults 30 years of age and older in the United States. *J Periodontol* 1999;**70**:13-29.

2. Black S, Kushner I, Samol D. C-reactive protein. *J Biochem* 2004; **279**:48487-484905.

3. Glurich I, Grossi S, Albini B. Systemic inflammation in cardiovascular and periodontal disease: A comparative study. *Clin Diagn Lab Immunol* 2002; **9**: 425–432.

4. Mattila K, Vesanen M, Valtonen V, Nieminen M, Palosuo T, Rasi V, *et al*. Effect of treating periodontitis on C-reactive protein levels: A pilot study. *BMC Infect Dis* 2002;**2**:30.

5. Herzberg M, Brintzenhofe K, Clawson C. Aggregation of human platelets and adhesion of *streptococcus sanguis*. *Infect Immun* 1983;**39**:1457-1469.

6. Herzberg MC. Effect of oral flora on platelets: possible consequences in cardiovascular diseases. *J Periodontol* 1996;67:1138-1142.

7. Beck JD, Slade G, Offenbacher S. Oral diseases cardiovascular diseases and systemic inflammation. *J Periodontol* 2000;**23**:110-120.

8. 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**:1528–34.

9. Loos BG. Systemic markers of inflammation in periodontitis. *J Periodontol* 2005;**76**:2106–15.

10. Iwamato Y, Nishimura F, Soga Y, Takeuchi K, Kurihara M, Takashiba S, *et al*. Antimicrobial periodontal treatment decreases serum C-reactive protein, tumor necrosis factor alpha, but not adiponectin in patients with chronic periodontitis. *J Periodontol* 2003;**74**:1231–6.

11. Yamazaki K, Honda T, Oda T, Ueki-Maruyama K, Nakajima T, Yoshie H, Seymour GJ. Effect of periodontal treatment on the C-reactive protein and proinflammatory cytokine levels in Japanese periodontitis patients. *J Periodontal Res* 2005;40:53-8.

12. Lalla E, Kaplan S, Yang J, Roth GA, Papapanou PN, Greenberg S. Effects of periodontal therapy on serum C- reactive protein, sE-selectin and tumor necrosis factor-alpha secretion by peripheral blood-derived macrophages in diabetes: a pilot study. *Journal of Periodontal Research* 2007; **42**: 274–282.

13. Al-Zahrani MS. Effect of periodontal treatment on serum C-reactive protein level in obese and normal-weight women affected with chronic periodontitis. *Saudi Med J* 2012; **33**:309-14.

14. Yazdi FK, Karimi N, Rasouli M, Roozbeh J. Effect of nonsurgical periodontal treatment on C-reactive protein levels in maintenance hemo dialysis patients. *Renal Failure* 2013; **35**:711-717.

15. Lagrand WK, Niessen HWM, Wolbink GJ. C-reactive protein colocalizes with complement in human hearts during acute myocardial infarction. *Circulation* 1997; **95**: 97-103.