

# Role of Systemic Markers in Periodontal Diseases: A Possible Inflammatory Burden and Risk Factor for Cardiovascular Diseases?

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## Abstract

**Background:** Periodontitis is a local inflammatory process mediating destruction of periodontium triggered by bacterial insult leading to systemic inflammatory mayhem in the host. Epidemiologically, it has been modestly associated with cardiovascular diseases (CVD) with elevated acute-phase reactant C-reactive protein (CRP) and rheological variables such as total leukocyte count and differential leukocyte count (TLC and DLC), which are potential predictors of CVD. **Aim:** The aim of this study was to investigate the serum CRP level, leukocyte count in chronic periodontitis patients and their relation to the severity of chronic periodontitis. **Subjects and Methods:** This cross-sectional study comprised 30 subjects, of which 20 were diagnosed as chronic periodontitis based on the Gingival index, probing depth and clinical attachment levels and 10 healthy subjects as controls. Following, which peripheral blood samples were drawn and serum CRP, TLC and DLC were quantified using the turbidimetric immunoassay. Data was analyzed using Intercooled Stata 9.2 version, (Stata corporation, LP, USA) ANOVA, Mann Whitney U test and Newman-Keuls *post hoc* procedures. *P* values less than) 0.05 were considered as significant **Results:** The mean serum CRP levels were statistically significant ( $P < 0.05$ ) in severe and moderate periodontitis subjects when compared with healthy controls. Leukocytes were significantly elevated in severe periodontitis compared with moderate periodontitis and controls; this finding was primarily explained by the increase in number of neutrophils. **Conclusion:** The increased serum CRP levels and neutrophils in chronic periodontitis subjects suggest an addition to the inflammatory burden of the individual potentially striking toward an increasing risk for cardiovascular events. Further research is needed to determine the specificity of these markers and their role in the inflammatory burden of one's systemic health.

**Keywords:** Cardiovascular disease, C-reactive protein, Leukocytes, Periodontitis, Risk factor

## Introduction

Periodontitis is a bacterially induced, localized, chronic inflammatory disease that destroys the connective tissue and the bone supporting the teeth.<sup>[1]</sup> It is a prototype of low grade local infection associated with a moderate systemic inflammatory response. Patients suffering from severe forms of this disease seem to have perturbation of their systemic

homeostasis. Periodontitis is characterized by a non-specific acute-phase response with exhibition of strong acute-phase reactants like C-reactive protein (CRP).<sup>[2]</sup> Loos<sup>[3]</sup> reported that periodontitis may also affect the cellular components of blood with slight elevation in the number of leukocytes when compared with controls. These same systemic markers appear to be predictive of atherosclerosis, myocardial infarction, stroke and thrombosis.

Epidemiological associations between periodontitis and cardiovascular disease (CVD) have been reported.<sup>[4,5]</sup> Both conditions have complex etiologies, genetic and gender predispositions and might share similar pathogenic mechanisms as well as common risk factors.<sup>[6]</sup> It is becoming increasingly clear that infections and chronic inflammatory conditions like periodontitis may influence the atherosclerotic process.<sup>[7]</sup>

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Pearson *et al.*<sup>[8]</sup> in a recent joint consensus conference of the American Heart Association and the Center for Diseases Control has identified different risk categories based on serum CRP levels. Subjects with CRP concentrations less than 1 mg/l are considered to be at low risk, whereas those with concentrations in the 1-3 mg/l range are assigned a medium risk level and those with more than 3 mg/l in serum CRP are considered to be at high risk for future CVD and events. Paraskevas *et al.*<sup>[9]</sup> in a systematic review and meta-analysis drew upon the conclusion that there is convincing evidence that plasma CRP was elevated in periodontitis affected patients compared with controls. It has been hypothesized that any association between periodontitis and CVD could be attributed to the moderate increases in CRP reported in subjects with poor periodontal health.<sup>[10]</sup>

A number of epidemiological studies consistently have shown a significant relationship between white blood cell (WBC) count and the occurrence of CVD and stroke.<sup>[11-13]</sup> Kweider *et al.*<sup>[14]</sup> in his study showed that chronic periodontal infections are associated with increases in number of leukocytes and Beck *et al.*<sup>[15]</sup> reported that increase in leukocyte count would modify the blood rheology thereby promoting hypercoagulation.

The aim of this study was first to determine serum concentrations of established risk markers of atherosclerosis such as CRP and peripheral blood markers such as total leukocyte count and differential leukocyte count (TLC and DLC) in chronic periodontitis and observe their relation to the severity of periodontal disease.

## Subjects and Methods

### Study population

A total of 30 systemically healthy subjects aged between 30 and 60 years, who reported to the out-patient section, Department of Periodontics, P. M. N. M Dental College and Hospital, Bagalkot were selected randomly during August to October 2009 (3 month short-term research). The study was approved by the Ethical Committee of the Institution. The protocol was clearly explained to patients and informed consent was obtained from all the recruits. The study was in accordance with the Helsinki Declaration of 1975 as revised in 2002.<sup>[16]</sup>

Individuals with no history of CVD or other acute/chronic systemic disorders were included in the study. Pregnant women, lactating mothers and individuals with trauma or who underwent recent tooth extractions or who had received periodontal and antimicrobial therapy in last 3 months before sampling was excluded from the study. Smoking increases WBC count mainly because of an increase in the number of neutrophils, which also occurs in periodontitis.<sup>[17]</sup> Therefore, smokers were excluded.

### Periodontal parameters

A single examiner (SE) performed the periodontal examination for all the recruited subjects for Gingival index (GI) (Loe and Silness 1963)<sup>[18]</sup>, probing depth (PD) and clinical attachment level (CAL). Both PD and CAL were measured by graduated William's periodontal probe on four sites of all present teeth except the third molars. PD and CAL nearest to the lower values were considered. Depending upon the PD and CAL measurements, study subjects were divided into three groups:

- Group I ( $n = 10$ ): Moderate chronic periodontitis; PD = 4-6 mm, CAL = 3-4 mm
- Group II ( $n = 10$ ): Severe chronic periodontitis; PD > 6 mm, CAL  $\geq$  5 mm
- Group III ( $n = 10$ ): Control group; PD < 3 mm, CAL < 3 mm.

Subjects were considered controls according to NHANES-III-1988-94.<sup>[19]</sup> CAL is an age-dependent variable and patients with CAL < 3 mm were reported to be present in more than 50/100% of population.

### Serum sampling and laboratory analysis

A total volume of 5 ml of non-fasting venous blood sample was collected and transferred into ethylenediaminetetraacetic acid (EDTA) coated collecting tubes and sent to the laboratory to measure TLC and serum CRP. Serum CRP levels were quantified using turbidimetric immunoassay (TURBILYTE-CRP, Tulip diagnostics, Goa, India). The detection limit of serum CRP was 0.5 mg/l while the reference range was < 0.5-22 mg/l.

### Statistical analysis

The statistical data were analyzed using Intercooled Stata 9.2 version, (Stata Corporation, LP, USA). The results were presented as mean and standard deviation. Overall differences among the three groups for all variables including GI, PD, CAL, CRP, total leukocyte counts (TLC) and neutrophils were determined by ANOVA. The pairwise differences among the three groups were carried by Mann-Whitney U test and Newman-Keuls *post hoc* procedure. The  $P < 0.05$  was considered to be statistically significant.

## Results

This cross-sectional study includes total of 30 patients divided into three groups. Group I Group II Group III [Table 1]. The inter-group comparison of periodontal parameters was carried out by ANOVA test [Table 2]. The differences between the three groups were significant ( $P < 0.001$ ) in terms of GI scores, PD and CAL [Table 1]. The mean GI score was statistically significant when Group II was compared with Group I ( $P = 0.03$ ). Comparison of mean GI scores showed that the differences between Groups I and II were significant when compared with those of Group III ( $P < 0.001$ ) [Table 1]. Likewise, pairwise comparisons of PD and CAL among the three groups were highly significant ( $P < 0.001$ ) [Table 2].

**Table 1: Study population data, periodontal and CVD parameters**

Characteristics	Group I	Group II	Group III	P value group II versus group III
Age	44 (5.8)	47.5 (5.3)	34.4 (4.6)	
Male/female %	70/30	80/20	60/40	
GI score	1.7 (0.43)	2.17 (0.15)	0.82 (0.3)	<0.001*
PD (mm)	5.33 (0.4)	7.11 (0.65)	1.98 (0.38)	<0.001*
CAL (mm)	4.21 (0.43)	7.2 (0.69)	1.2 (0.33)	<0.001*
CRP (mg/l)	4.48 (0.85)	6.65 (1.4)	2.45 (0.58)	<0.001*
TLC (per mm <sup>3</sup> )	7310 (858.23)	9090 (1530.03)	5500 (778.89)	<0.001*
Neutrophil count (%)	65.9 (9.72)	70.1 (4.77)	54.4 (6.17)	<0.001*

GI: Gingival index, PD: Probing depth, CAL: Clinical attachment levels, CRP: C-reactive protein, TLC: Total leukocytic count, CVD: Cardiovascular diseases, \*Statistically significant ( $P < 0.05$ )

**Table 2: Pair wise comparison of mean periodontal and CVD parameters using Newman-Keuls post hoc procedure**

Blood Parameters (mean)	Group III versus group I	Group III versus group II	Group I versus group II
GI	$P=0.0002^*$	$P=0.0002^*$	$P=0.03^*$
PD	$P=0.0001^*$	$P=0.0001^*$	$P<0.001^*$
CAL	$P=0.0001^*$	$P=0.0001^*$	$P<0.001^*$
CRP	$P=0.0002^*$	$P=0.0001^*$	$P<0.001^*$
TLC	$P=0.0012^*$	$P=0.0001^*$	$P<0.001^*$
Neutrophil count	$P=0.0015^*$	$P=0.0002^*$	$P=0.20$

GI: Gingival index, PD: Probing depth, CAL: Clinical attachment levels, CRP: C-reactive protein, TLC: Total leukocytic count, CVD: Cardiovascular diseases, \*Statistically significant ( $P < 0.05$ )

A significant difference in mean CRP serum level was observed over all study groups ( $P < 0.001$ ) [Table 2]. The TLC among the three groups was significantly different ( $P < 0.001$ ) [Table 2]. The severe periodontitis group had higher mean number of leukocytes compared with moderate periodontitis patients and control group (9090/mm<sup>3</sup> vs. 7310/mm<sup>3</sup> and 5500/mm<sup>3</sup> respectively). While the other types of leukocytes did not vary significantly, a significant difference in number of neutrophils was observed over the 3 study groups [Table 2].

Pairwise comparisons of mean CRP level and TLC among three groups were statistically significant. The comparison of mean neutrophil count was significant when Group III was compared with Groups I and II [Table 2]. However, pairwise comparison of mean neutrophil score was not significant when Group I was compared with Group II [Table 2].

## Discussion

Cross-sectional as well as prospective studies have established that levels of systemic markers of inflammation including CRP, fibrinogen, Interleukin-6 (IL-6) are associated with the pathogenesis of atherosclerosis and the risk of CVD.<sup>[20-22]</sup> CRP in particular has been the focus of attention as a key marker of atherosclerosis and elevated level ( $\geq 2.1$  mg/l) constitute a risk predictor of CVD.<sup>[13,23]</sup> In addition, leukocyte count is considered as a good predictor of ischemic heart disease.<sup>[12]</sup>

It has been proposed that these markers could be elevated in undiagnosed chronic infectious processes like periodontitis. It is conceivable that chronically, elevated systemic markers in blood exacerbate other ongoing inflammatory processes in other organ systems and this way perhaps increase the risk for atherosclerosis leading to cardiovascular and cerebrovascular events.<sup>[24]</sup>

In this reverence, CRP could be a sensitive systemic marker used to evaluate the inflammatory status of an individual.<sup>[10]</sup> Chronic bacterial infections like periodontitis are one of the established risk factors for moderately elevated CRP levels.<sup>[25]</sup>

In the present study, we observed a significant association between periodontitis and CRP. Moreover, the results showed a positive correlation between the severity of periodontal disease and CRP level. The findings of the current study support previous studies, which reported similar results.<sup>[25-28]</sup> Noack *et al.*<sup>[27]</sup> observed statistically significant increases in CRP in subjects with moderate to severe periodontitis when compared with healthy controls after adjusting for potential confounding factors. The presence of periodontal pathogens including *Porphyromonas gingivalis*, *Prevotella intermedia* and *Tannerella forsythensis* from subgingival plaque samples was also positively associated with elevated CRP levels.<sup>[27]</sup>

Elevated the numbers of leukocytes in periodontitis have previously been observed.<sup>[14]</sup> Fredriksson *et al.*<sup>[17]</sup> reported slight elevation of leukocytes in periodontitis in comparison with controls, though the results were not statistically significant. Kowolik *et al.*<sup>[29]</sup> showed an elevated leukocyte count and more specifically, neutrophil count, is a good predictor for subsequent coronary heart disease. The data obtained from our study showed significantly increased TLC in periodontitis as compared with healthy controls, which is in agreement with the results of other studies and study carried out by Loos *et al.* who found a significant increase in polymorphonuclear leukocytes.<sup>[30]</sup> The increase in number of leukocytes in periodontitis has been suggested to be mainly due to an increase in the number of polymorphonuclear leukocytes.<sup>[29]</sup> In a large-scale follow-up study carried out by Twig *et al.*<sup>[31]</sup> demonstrated that a single measurement of WBC in healthy young men may predict coronary artery disease (CAD) incidence independently from

other risk factors for CAD such as elevated lipids and a positive family history. It has also been suggested that the higher numbers of leukocytes increase the blood rheology thereby increasing the risk for CVD.<sup>[12]</sup>

The elevated levels of CRP, neutrophils in patients with periodontitis may occur when bacteria and bacterial products, such as lipopolysaccharides as well as locally produce pro-inflammatory cytokines enter the circulation.<sup>[30]</sup>

There are few limitations in the current study. The sample size was small; long-term studies with larger sample size as well as large prospective studies are therefore needed to further elucidate the interrelationship between periodontal disease, CRP and CVD. This would place us in a position to give more rational advice to at-risk patients with chronic periodontitis, particularly in relation to possible prevention of CVD.

## Conclusion

In summary, our study provides evidence that periodontitis may cause systemic changes in plasma levels of CRP and numbers of leukocytes and neutrophils. These changes are positively correlated with the severity of periodontitis. Thus, periodontitis, a common chronic condition, predisposes affected patients to CVD by increasing the levels of systemic markers of inflammation, which may contribute to the process of atherosclerosis. Therefore, periodontitis deserves serious consideration as a risk factor for CVDs. Further confirmatory investigations are needed to study this inflammatory burden.

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