

Estimation of the Levels of C-reactive Protein, Interleukin-6, Total Leukocyte Count, and Differential Count in Peripheral Blood Smear of Patients with Chronic Periodontitis in a South Indian Population

DK Gani¹, SK Mallineni², Ambalavanan³, Ramakrishnan³, Deepalakshmi³, P Emmadi³

ABSTRACT

Aims: The aim of the present study is to investigate systemic levels of inflammatory markers of cardiovascular diseases like C-reactive protein (CRP), interleukin-6 (IL-6), total leukocyte count and differential count in patients with chronic periodontitis, in comparison to healthy individuals without periodontal disease.

Subjects and methods: A total of 42 individuals, both males and females, above the age of 30 years, were included. Healthy controls (Group I, n = 14), patients with chronic localized periodontitis (Group II, n = 14) and chronic generalized periodontitis (Group III, n = 14), all without any other medical disorder were recruited and peripheral blood samples were taken. Serum samples of CRP and IL-6 were estimated by using different techniques. Total leukocyte count and differential count were estimated by standard clinical laboratory method.

Results: Groups II and III had higher mean CRP levels than Group I (0.479, 0.544 versus 0.304 mg/dL). C-reactive protein level in Group III was statistically significant when compared to Group I (p = 0.04). Group III had higher median IL-6 level (6.35 pgm/ml) than Group II (< 5.0 pgm/ml) and Group I (< 5.0 pgm/ml). Median values of IL-6 were not statistically significant in any group (p = 0.29). Total leukocyte count was also elevated in Group III ($10.4 \times 10^3/c.mm$) compared to Group II and Group I ($9.2 \times 10^3/c.mm$ and $7.9 \times 10^3/c.mm$). This was statistically significant between different study groups (p < 0.0001). Neutrophil count in Group III was higher (68.0%) than Group II (62.4%) and Group I (57.4%). Neutrophil percentage was statistically significant in Group III, when compared to Group I (p = 0.0003).

Conclusion: Periodontitis results in higher systemic levels of CRP, IL-6, total leukocyte count and neutrophils. These elevated inflammatory factors may increase inflammatory activity in atherosclerotic lesions, potentially increasing the risk for cardiovascular events.

Keywords: Cardiovascular disease, C-reactive protein, differential count, inflammatory markers, interleukin-6, periodontitis, peripheral blood smear, South Indian population

Estimado de los Niveles de Proteína C-reactiva, Interleucina-6, Conteo Total de Leucocitos, y Conteo Diferencial en el Frotis Sanguíneo Periférico de Pacientes con Periodontitis Crónica en la Población del Sur de la India

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RESUMEN

Objetivos: El objetivo del presente estudio es investigar los niveles sistémicos de los marcadores inflamatorios de enfermedades cardiovasculares, como la proteína C-reactiva (PCR), la interleucina-6 (IL-6), el conteo total de leucocitos, y el conteo diferencial en los pacientes con periodontitis crónica, en comparación con individuos saludables sin la enfermedad periodontal.

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Sujetos y métodos: El estudio comprendió un total de 42 individuos – varones y hembras – mayores de 30 años de edad. Los controles saludables (Grupo I, $n = 14$), los individuos con periodontitis crónica localizada (Grupo II, $n = 14$), y aquellos con periodontitis crónica generalizada (Grupo III, $n = 14$), todos sin ningún otro problema médico, fueron reclutados y se tomaron muestras de sangre periférica. Las muestras de suero de PCR e IL-6 fueron estimadas usando técnicas diferentes. El conteo total de leucocitos y el conteo diferencial fueron calculados mediante el método estándar de laboratorio clínico.

Resultados: Los grupos II y III tuvieron niveles promedio más altos de PCR que el Grupo I (0.479, 0.544 frente a 0.304 mg/dL). El nivel de proteína C-reactiva en el Grupo III fue estadísticamente significativo, comparado con el Grupo I ($p = 0.04$). El Grupo III tuvo un nivel mediano más alto de IL-6 (6.35 pgm/ml) que el Grupo II (< 5.0 pgm/ml) y el Grupo I (< 5.0 pgm/ml). Los valores medianos de IL-6 no fueron estadísticamente significativos en ningún grupo ($p = 0.29$). El conteo total de leucocitos también fue elevado en el Grupo III ($10.4 \times 10^3/c.mm$) comparado con el Grupo II y el Grupo I ($9.2 \times 10^3/c.mm$ y $7.9 \times 10^3/c.mm$). Dicho conteo fue estadísticamente significativo entre diferentes grupos de estudio ($p < 0.0001$). El conteo de neutrófilos en el Grupo III fue más alto (68.0%) que en el Grupo II (62.4%) y el Grupo I (57.4%). El porcentaje de neutrófilos fue estadísticamente significativo en el Grupo III, en comparación con el Grupo I ($p = 0.0003$).

Conclusión: La periodontitis produce niveles sistémicos más altos de PCR, IL-6, conteo total de leucocitos y neutrófilos. Estos factores inflamatorios elevados pueden aumentar la actividad inflamatoria en las lesiones ateroscleróticas, aumentando potencialmente el riesgo de accidentes cardiovasculares.

Palabras claves: Cardiovascular disease, C-reactive protein, differential count, inflammatory markers, interleukin-6, periodontitis, peripheral blood smear, South Indian population

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INTRODUCTION

Although periodontitis is a chronic inflammatory disease, a number of hypotheses have proposed that it has an aetiological or modulating role in cardiovascular disease (CVD). Periodontitis is a chronic infection of the supporting tissues of the teeth. It is estimated that about 15% of adults from 21 to 50 years and about 30% of subjects > 50 years have severe periodontitis (1). Importantly, based on cross-sectional and prospective epidemiological studies, periodontitis has been linked to cardiovascular diseases and cerebrovascular ischaemia, although mechanisms responsible for this association are obscure (2). Several parameters of systemic inflammation have been identified as for example, levels of C-reactive protein (CRP) in the range 1 to 3 mg/L have recently gained special attention as risk factors for cardiac and cerebrovascular events (2, 3). Also, elevated plasma levels of interleukin-6 (IL-6) have been associated with unstable angina and cardiovascular diseases and IL-6 is related to other cardiovascular risk factors (3). Moreover, it has been established that IL-6 induces CRP production (4). Furthermore, increased number of leukocytes has been associated with cardiovascular diseases. It has been proposed that elevated CRP, IL-6 and leukocytes in patients with cardiovascular disease may be the result of chronic infectious and inflammatory processes, in particular *Helicobacter pylori*, *Chlamydia pneumoniae* and cytomegalovirus (CMV) infections (1–5).

Although a number of studies have been performed recently on the possible association between CVD and perio-

odontitis, this association is not thoroughly understood so far. Despite the fact that many studies have shown associations that link periodontal disease to cardiovascular disease; there have been relatively few studies to address the potential effects of periodontal treatment on surrogate markers of cardiovascular risk or cardiovascular outcomes. Recent studies have suggested that periodontal treatments can reduce levels of serum high sensitivity-CRP, lower IL-6 and improve endothelial function (6–9) as measured by flow-mediated dilation. It is not known whether the vascular lesions that are associated with periodontal disease are fully reversible. Indeed, not all multifactorial diseases can be reversed by removing an aetiological component. This is particularly relevant for chronic diseases in which the causative agent was present, perhaps insidiously, for decades prior to clinical onset.

The purpose of the present study is to evaluate the levels of C-reactive protein, interleukin-6, total leukocyte count and differential count in patients with chronic generalized and localized periodontitis as compared to healthy individuals without periodontal disease.

SUBJECTS AND METHODS

Patients for the study were selected from the Department of Periodontics, Meenakshi Ammal Dental College and Hospital. The sample size of this study was 42 patients above 30 years of age. Among them, 28 patients diagnosed as having chronic periodontitis with probing pocket depth ≥ 5 mm and radiographic evidence of bone loss were included in the study, and 14 healthy individuals without periodontal disease

were included as controls. A written informed consent was obtained from all subjects in the study prior to examination. The study was approved by an Institutional Human Ethical Committee.

Exclusion criteria included smoking, pregnant women, individuals with acute or chronic medical disorders including diabetes, viral, fungal or bacterial infections, trauma and recent tooth extractions, and patients with less than 27 teeth. The patients were divided into three groups based on clinical attachment level (CAL). Group I (Controls) consisted of 14 individuals who were free from periodontal disease. Group II consisted of 14 patients who were diagnosed as having chronic localized periodontitis. Group III consists of 14 patients who were diagnosed as having chronic generalized periodontitis (criteria followed for diagnosing periodontitis have been published [10]). Patients with > 30% of sites with loss of attachment were classified as having generalized periodontitis, and patients with < 30% of sites with attachment loss were classified as having localized periodontitis. Non-fasting venous blood was collected from each subject in EDTA containing tubes. One tube was analysed within three hours in a clinical laboratory for standard measurements of the total number of leukocytes and leukocyte differentiation. Another tube was immediately put on ice, centrifuged and serum prepared within two hours. Serum samples were stored in a storage box containing dry ice pack, before being transferred and stored in a freezer at -70°C. Serum levels of CRP were determined using particle enhanced turbidimetric immunoassay (PETIA) technique (11). The reference range in this method is 0.2 – 0.3 mg/dL. In this method, latex particles coated with antibody to CRP aggregate in the presence of C-reactive protein in the sample. The increase in turbidity which accompanies aggregation is proportional to the CRP concentration. Serum levels of IL-6 were determined using chemiluminescent immunoassay (IMMULITE) technique (12). Reference range in this method is non-detectable to 5.9 pgm/ml. The IL-6 test unit contains one bead, coated with a monoclonal murine anti-IL-6 antibody, which aggregates in the presence of IL-6 in the sample.

Mean and standard deviation were estimated from the sample for each study group. Mean values were compared by one-way ANOVA. Multiple range tests by Tukey – HSD procedure were employed to identify the significant groups at 5% level. Median and range were estimated for certain parameters wherever it was appropriate. Median values were compared between different study groups by median test (non-parametric procedure).

Categorical variables were compared between groups by Pearson’s Chi-square test. Pearson’s correlation analysis/ Spearman rank correlation was done appropriately to assess the relationship between various parameters in each study group. In the present study, $p < 0.05$ was considered as the level of significance.

RESULTS

Forty-two patients were divided into three Groups with 14 individuals each. A single examiner conducted the periodontal assessment in order to minimize the variation in the data. Groups II and III had higher mean CRP levels than Group I (0.479, 0.544 *versus* 0.304 mg/dL). C-reactive protein level in Group III was statistically significant when compared to Group I ($p = 0.04$) [Table 1, Fig. 1]. Group III had

Table 1: The mean ± standard deviation and test of significance of mean values between different study groups for C-reactive protein (CRP)

Parameter	Group	Median ± SD	p-value*	Significant group at 5% level
CRP	I	0.304 ± 0.073		
	II	0.479 ± 0.339		III vs I
	III	0.544 ± 0.264	0.04 (Sig)	

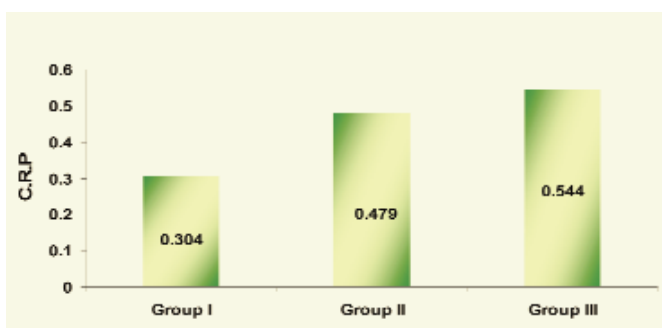


Fig. 1: Comparison of C-reactive protein (CRP) mean values in different study groups.

higher median IL-6 level (6.35 pgm/ml) than Group II (< 5.0 pgm/ml) and Group I (< 5.0 pgm/ml). Median values of IL-6 were not statistically significant in any Group ($p = 0.29$) [Table 2]. Total leukocyte count was also elevated in

Table 2: The median, range and test of significance of median values between different study groups for interleukin-6 (IL-6)

Parameter	Group	Median (Range)	p-value*	Significant group at 5% level
IL - 6	I	< 5.0 (< 5.0 – 15.9)		
	II	< 5.0 (< 5.0 – 14.4)		
	III	6.35 (< 5.0 – 22.4)	0.29 (NS)	Nil

Group III ($10.4 \times 10^3/c.mm$) compared to Groups II and Group I ($9.2 \times 10^3/c.mm$ and $7.9 \times 10^3/c.mm$) [Table 3]. This was a statistically significant difference between different study groups ($p < 0.0001$). Neutrophil count in Group III was

Table 3: The mean and standard deviation of total leukocyte count (per cmm) in different study groups

Patient No	Group I	Group II	Group III
1	9.400	9.800	9.800
2	8.800	11.000	9.000
3	6.800	9.600	10.000
4	8.600	8.900	10.200
5	7.600	7.800	9.800
6	8.000	9.700	12.000
7	8.700	8.800	9.800
8	7.200	8.800	10.400
9	6.500	9.700	12.100
10	8.000	9.600	11.000
11	8.500	8.300	12.200
12	6.700	9.800	9.800
13	7.800	8.800	10.200
14	8.400	8.800	8.800
Mean \pm SD	7900 \pm 0.9	9200 \pm 0.8	10400 \pm 1.1

higher (68.0%) than Group II (62.4%) and Group I (57.4%). Neutrophil percentage was statistically significant in Group III, when compared to Group I ($p = 0.0003$). The comparison between total leukocytes and neutrophils is summarized in Fig. 2. However, comparison between CRP and total

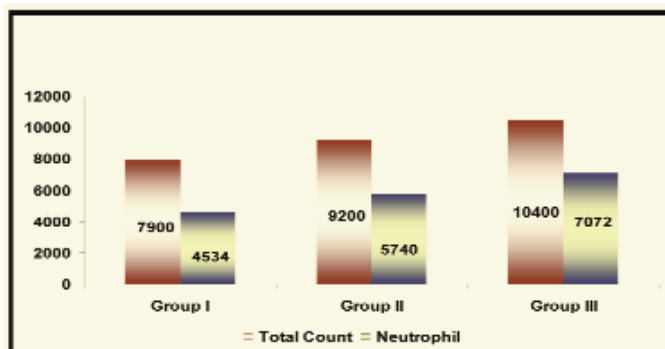


Fig. 2: Comparison of total leukocytes count and neutrophils count in different study groups.

counts showed that there was no statistically significant correlation between any of the three groups. There was a statistically significant correlation between CRP levels and the neutrophil count in the chronic generalized periodontitis group alone, rather than the other two groups.

DISCUSSION

Periodontitis is a chronic infection affecting the tissues surrounding and supporting the teeth and is related to bacteria in the dental plaque and the inflammatory response of the host. Furthermore, in the last decade, there have been numerous case-control and longitudinal studies that generally, but not unanimously, support a statistically significant association between the clinical presentation of periodontal disease and more severe CVD. Since CVD is one of the most common causes of death in developed countries, understanding the risk factors for atherosclerosis and CVD has been a very

active area of investigation. In the last decade, researchers have shifted their focus from classic causes to alternative risk factors. Now it is well known that atherosclerosis occurs in response to an injury of the vascular endothelium and that it is an inflammatory process (13). A hypothesis of the contribution of chronic infections to atherogenesis led scientists to investigate the relationship between oral hygiene and CVD (2–9). Epidemiological studies suggest a link between periodontal infections and an increased risk for CVD, and associations are observed between the severity of periodontitis and coronary atherosclerosis. Nevertheless, periodontitis is a common, often-undiagnosed chronic infection of the supporting tissues of the teeth. Persistent infections such as periodontitis induce inflammatory and immune responses which may contribute to coronary atherogenesis and, in conjunction with other risk factors, may lead to coronary heart disease. Cardiovascular diseases are a heterogeneous group of conditions that have significant morbidity and mortality. During the last decade, poor dental health has been found to be significantly CVD (1–5) and a significant association between periodontal disease and coronary heart disease was reported in the literature (14). However, one postulated the evidence for inflammation playing a role in the pathogenesis of CVD (2). Some inflammatory or haemostatic markers constitute cardiovascular risk factors. In this respect, the acute phase reactant CRP is of special interest. C-reactive protein represents an emerging and reliable marker of the acute phase response to infectious burdens and/or inflammation. The causal link between CRP and CVD remains to be established. The association of CRP with CVD may be attributed to its association with other reactant proteins that promote thrombosis. However, as a consequence of its kinetics, it best describes the inflammatory status of the individual (15). C-reactive protein hepatic production is usually elicited by an inflammatory stimulus and mediated through a complex network of cytokines, mainly IL-6 (16). C-reactive protein has also assumed a significant role as a predictor for future coronary events in healthy populations (17). Interleukin-6 is an inflammatory cytokine, released from monocytes, lymphocytes or endothelial cells at sites of tissue injury. Interleukin-6 stimulates release of neutrophils and platelets from bone marrow into circulating blood, a part of acute and chronic inflammatory reactions. Increased total blood leukocyte (and neutrophil count) may promote atherosclerosis, thrombosis, and ischaemia through several potential mechanisms, including microvascular occlusion by activated neutrophils and monocytes under ischaemic conditions. It has been shown that leukocyte count is a significant predictor of coronary heart disease risk, independent of classic coronary risk factors.

In this study, the mean level of CRP in the chronic generalized periodontitis group was 0.544 mg/dL, in chronic localized periodontitis group, it was 0.479 mg/dL and in the control group, it was 0.304 mg/dL. Comparison among these three groups showed increased mean CRP in patients with

chronic generalized periodontitis, which was statistically significant. This is in accordance with the studies which were reported in the literature (2, 3, 17–19). The normal range of CRP in healthy individuals (without periodontal diseases) was noted as 0.2–0.3 mg/dL. C-reactive protein value > 0.3 mg/dL is considered indicative of high risk for developing atherosclerotic CVD. It is interesting to note that in the present study, CRP levels in both test groups indicated that patients with either chronic generalized or chronic localized periodontitis may be at higher risk for atherosclerotic CVD. The median levels of IL-6 in the chronic generalized periodontitis group were 6.35 pgm/ml and it was < 5.0 pgm/ml in both the chronic localized periodontitis and control groups. The median level of IL-6 in the chronic generalized periodontitis group was higher than the other groups, but was not statistically significant. However, few studies reported that periodontitis patients have higher CRP and IL-6 levels when compared with the periodontally healthy population (3, 20–23). The total leukocyte count in the chronic generalized periodontitis group had higher mean leukocytes than the other two groups, which was statistically significant (24, 25). This was mainly due to the difference in the number of neutrophils which increased and lymphocytes which decreased, while the other types did not vary significantly. It may be speculated that periodontitis may predispose affected patients to cardiovascular diseases by increasing the levels of acute phase proteins which may lead to increased inflammatory activity in atherosclerotic lesions. However, the limitations of this study including sample size and randomization of the sample should be considered.

CONCLUSIONS

The relationship between periodontal disease and CVD is a scientifically established interplay that has its basis in altered immunologic mechanisms. The ability to predict cardiovascular risk markers in periodontal disease would therefore have immense value in the prevention and treatment of such disease.

The following conclusions were drawn:

- Mean CRP level in the chronic generalized periodontitis group was higher, when compared to the control group and it was also statistically significant.
 - Even though the mean CRP level in the chronic localized periodontitis group was higher when compared to controls, no statistically significant difference was found.
 - Comparison between chronic generalized and chronic localized periodontitis groups also had no statistical significance in spite of the former group having higher CRP levels.
 - The median level of IL-6 was raised in the chronic generalized periodontitis group than in the other two groups, but it was not statistically significant.
- The total leukocyte count and neutrophil count were also increased in both chronic localized and chronic generalized periodontitis groups than the control group.
 - The total leukocyte count was statistically significant in the chronic generalized periodontitis group, when compared to other groups. Neutrophil count was statistically significant in the chronic generalized periodontitis group, when compared to the control group.

Thus it may be concluded from the present study that CRP and IL-6 could be risk markers for CVD in the study population. Further research focussing on a larger sample size is needed to establish these findings.

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