

Estimation of Serum Levels of C - reactive protein in Periodontitis Patients – An Observational Study

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Abstract

Original Research Article

Background: C-reactive protein (CRP) is an emerging prognostic biomarker in patients with periodontitis and cardiovascular disease. There is a proposed pathophysiological mechanism that links periodontitis with cardiovascular disease. Early detection of periodontitis patients with cardiovascular disease events helps in accurate diagnosis and better treatment plan. But there are limited studies in the literature on serum C- reactive protein levels in periodontitis patients. **Aim:** This observational study aimed to investigate the associations between periodontitis severity and the serum levels of CRP in comparison with healthy and periodontitis group. **Materials & Methodology:** A total of 30 patients who were divided into two groups of study and control group consist of 15 patients in each group. Patients were selected based on inclusion and exclusion criteria. Blood sample collection was done by trained professional and serum samples were stored at -80 degree Celsius. The C-reactive protein levels were measured using a high-sensitivity enzyme-linked immunosorbent assay. **Results:** The mean value of 12923.41 in C-reactive protein (CRP) serum levels in gingivitis group with the standard deviation of 10272.22 was obtained with the p value of 0.01. The mean of 3994.86 in C-reactive protein (CRP) serum levels in periodontitis group with the standard deviation of 5110.81 was obtained with the p value of 0.01 with the p value of 0.01. Serum C-reactive protein levels showed statistically significant results when compared between the control and study group. **Conclusion:** There is a pathophysiological mechanism that links the cardiovascular diseases with periodontitis; the serum C-reactive protein levels are higher in periodontitis patients. The serum CRP levels and may be used as a cardiac biomarker in periodontitis patients.

Keywords: Serum, C-reactive protein, periodontitis, enzyme-linked immunosorbent assay.

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INTRODUCTION

Periodontal disease is an inflammatory disease primarily initiated in response to a specific group of bacteria and characterized by a complex host-biofilm interaction, Sanz M *et al.*, According to the World Health Organization, the severe form of periodontitis causes tooth loss in about 5–15% of the population worldwide with a prevalence rate of up to approximately 90% collectively and it is considered the sixth most common disease affecting humans. Aberrant immune-inflammatory responses determine a patient's susceptibility to developing periodontitis, which may be modified by a range of risk factors Seymour GJ *et al.*, Cardiovascular disease is the second most common cause of death worldwide.

Epidemiological studies suggest that periodontitis is independently associated with an increased risk of atherosclerotic cardiovascular disease (CVD). Given increasing data suggesting that chronic inflammatory conditions can increase the risk of cardiovascular disease (CVD), attention has been turned to periodontitis and its potential link to CVD. While a possible association between periodontitis and atherosclerotic CVD has been more recently recognized, the link between dental procedures and bacteremia, and the implications on valvular heart disease, dates as far back as the early 1900s. This ultimately led to guidelines for antibiotic prophylaxis prior to dental procedures and was the impetus for dental evaluation or “dental clearance” prior to heart valve surgery. It is challenging to prove that

periodontitis is the source of inflammation resulting in CVD, largely because those with significant periodontitis often have other potential causes of increased inflammation, such as diabetes and tobacco use, that can confound findings.

While a direct causal relationship between periodontitis and atherosclerotic CVD has not been established, it is hypothesized that periodontitis, which can trigger an inflammatory cascade in the oral cavity, could, in moderate to severe cases, trigger a similar systemic cascade as a result of transient bacteremia.

At least, two mechanisms have been proposed to explain the associations between these two diseases. First, subgingival plaque bacteria can translocate into the circulation and promote an inflammatory or immune response within the atherosclerotic plaque. Additionally, pro-inflammatory cytokines locally produced in periodontitis lesions can enter the bloodstream, resulting in systemic inflammation, which is characterized by elevated levels of inflammatory biomarkers, such as C-reactive protein (CRP).

CRP and inflammation plays a fundamental role in the atherosclerosis process in the pathophysiology of coronary artery disease may lead to novel therapies that target aspects of the inflammatory process smoldering within the atheroma. CRP reflects activation of the inflammatory system and play a role in the prediction of first coronary events in combination with other risk profile factors. Moderately elevated serum CRP (>2 mg/l) concentration is a systemic marker of inflammation and a documented risk factor for CVD in otherwise healthy persons. Elevated CRP values have also been associated with other diseases.

C-Reactive Protein as a biomarker provides a reflection of the underlying atherosclerotic burden or activity useful for reliable, accurate, and cost-effective information; and to predict future events and blood levels of CRP are now commonly used in clinical practice to improve vascular risk prediction in primary and secondary prevention in cardiovascular disease.

So, aim of the study is to investigate the associations between periodontitis severity and the serum levels of CRP in comparison with healthy and periodontitis group.

METHODS AND METHODOLOGY

30 patients reporting to the outpatient of Periodontology and Implantology, Faculty of dental sciences, RUAS. The ethical clearance for this study was obtained from the ethical committee of the institution and informed consent were taken from the patient. The study was conducted in the Dept. of Periodontology and Implantology, Faculty of dental sciences, Ramaiah university of applied sciences. The present study is an observational study design.

Subjects with age 18 to 65 yrs. Patients with periodontitis should have at least 20 teeth having pocket depth ≥ 5 mm and at least three sites in each quadrant with clinical attachment loss ≥ 4 mm (CAL) and radiographic evidence of bone loss. Patient with diabetes mellitus, Smokers, patients with autoimmune diseases or osteoporosis, users of antibiotics or non-steroidal anti-inflammatory drugs within the last 3 months and patients who were subjected to any periodontal therapy during the last year were excluded from the study.

Sample Collection

Periodontal examinations were performed using mouth mirror and UNC 15 probe. Individuals with at least six teeth present in the selected quadrants were included for periodontal examination. Blood samples were obtained by trained personal. Briefly 2 ml of venous blood was collected from the antecubital fossa by venipuncture using a 20 gauge needle with a 2 ml syringe. Blood sample were allowed to clot at room temperature and, after 1 hour, serum was separated from blood by centrifugation and 0.5 ml of extracted serum was transferred to 1.5 ml aliquots. Each aliquot was stored at -80 degree Celsius until required for analysis. CRP levels were determined using a high-sensitivity enzyme-linked immunosorbent assay (GENLISA™LISA, KRISHGEN BioSystems) (cat No#KBH1798, Ver3.0) with a detection limit of less than 20 pg/ml.

Statistical Analysis

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., were used to perform statistical analyses. Descriptive analysis of all the explanatory and outcome parameters will be done using mean and standard deviation for quantitative variables, frequency and proportions for categorical variables. Independent Student t Test was used to compare the expression of C-Reactive Protein between the groups. Pearson's correlation test was used to estimate the C-Reactive Protein and the clinical parameters in each study group. The level of significance [P-Value] was set at $P < 0.01$.

RESULTS

This observational study comprises study population of 30 patients, control group of 15 patients, study group of 15 patients of gingivitis and periodontitis respectively with mean (\pm SD) age of 48.7 ± 4.4 years (age range: 35–65) and with 65% of males and 35% of females. The C-reactive protein (CRP) serum levels in gingivitis group is shown in the (Table: 1). In the total of 15 patients the highest concentration of $3.243 \mu\text{g/L}$ and the lowest concentration of $2.007 \mu\text{g/L}$ was obtained (Table: 2) The mean of 12923.41 in C-reactive protein (CRP) serum levels in (control group) gingivitis group with the standard deviation of 10272.22 was obtained. With the p value of 0.01 (Table: 3).

The mean of 3994.86 in C-reactive protein (CRP) serum levels in (study group) periodontitis group with the standard deviation of 5110.81 was obtained with the p value of 0.01.

The C-reactive protein (CRP) serum levels in gingivitis group compared to the C-reactive protein (CRP) serum levels in periodontitis patients is given in the (table:3). C-reactive protein serum levels in gingivitis and C-reactive protein serum levels in periodontitis shows statistically significant difference with p value of 0.01.

CRP in gingivitis group showed less serum concentration than any other group. C-reactive protein serum concentration showed statistically significant results with the p value of 0.01.

Study group (periodontitis group) patients of serum levels of C-reactive protein levels in periodontitis patients, 5 patients showed positive correlation with the serum C-reactive protein levels. A total of 33% patients showed positive correlation between the serum C-reactive protein levels.

Table 1: CRP serum levels in Control group

Serum CRP levels in control group														
Serum 1	Serum 2	Serum 3	Serum 4	Serum 5	Serum 6	Serum 7	Serum 8	Serum 9	Serum 10	Serum 11	Serum 12	Serum 13	Serum 14	Serum 15
2.458	2.36	2.988	2.498	3.03	3.248	2.894	3.189	2.942	2.111	2.007	2.428	2.613	2.828	2.627
p value- 0.01*														

Table 2: CRP serum levels in study group

Serum CRP levels in study group														
Serum 1	Serum 2	Serum 3	Serum 4	Serum 5	Serum 6	Serum 7	Serum 8	Serum 9	Serum 10	Serum 11	Serum 12	Serum 13	Serum 14	Serum 15
2.667	1.965	2.03	1.999	2.337	2.159	2.238	3.021	2.378	2.019	1.841	1.781	1.781	2.349	1.346
p value- 0.01*														

Table 3: Showing the CRP serum levels in gingivitis and periodontitis group

Comparison of serum CRP levels in control and study group			
Control group	Serum CRP	Study group	Serum CRP
Serum 1	2.458	Serum 1	2.667
serum 2	2.36	serum 2	1.965
serum 3	2.988	serum 3	2.03
serum 4	2.498	serum 4	1.999
serum 5	3.03	serum 5	2.337
serum 6	3.243	serum 6	2.159
serum 7	2.894	serum 7	2.238
serum 8	3.189	serum 8	3.021
serum 9	2.942	serum 9	2.378
serum 10	2.111	serum 10	2.019
serum 11	2.007	serum 11	1.841
serum 12	2.428	serum 12	1.781
serum 13	2.613	serum 13	1.781
serum 14	2.828	serum 14	2.349
serum 15	2.627	serum 15	1.346
p value - 0.01*			

DISCUSSION

This observational study with 30 total sample size, divided into 15 patients of control group, 15 patients of study group assessed the CRP levels in serum. Although periodontitis is a chronic

inflammatory disease but some factors of acute inflammation phase are involved in this disease among which is the C-Reactive protein (CRP). CRP can also be used for the prediction and early detection of periodontal disease.

Majid feredyoni golpasha et al., conducted a study to compare the amount of salivary C-Reactive protein (CRP) in healthy subjects and patients with periodontal disease. The statistical analysis showed a significant difference in salivary CRP concentrations between the periodontitis patients and healthy subjects ($P=0.045$) which is in accordance with our study results.

Epidemiological studies have demonstrated that elevated CRP is associated with an increased risk for future cardiovascular events *Kaptoge et al.*, 2010., evidence suggests that CRP may be involved in several events of atherogenesis, such as endothelial dysfunction, oxidized low-density lipoprotein uptake by macrophages, and vascular smooth muscle cell proliferation *Devaraj, Singh, & Jialal.*, Study done by in group of Thai adults.

In one meta-analysis the findings resulted in a conclusion that periodontitis and poor oral health overall indeed contribute to the pathogenesis of cardiovascular disease *Meurman et al.*,

Serum levels of CRP in periodontitis patients when compared with the serum levels of CRP in gingivitis showed statistically significant difference. This is in accordance with the one more study done by *Kitti Torrungruang et al.*, where cross-sectional study examined the associations between periodontitis and the serum C-reactive protein (CRP)—in systemically healthy adults.

Demonstrated that periodontitis and poor oral hygiene were associated with increased serum levels of CRP in otherwise healthy individuals. Showed that the participants with poor oral hygiene, as indicated by higher plaque scores, were twofold more likely to have elevated serum CRP levels, compared to those with good oral hygiene.

Future studies assessing the association between periodontitis and cardiovascular diseases must also consider the prevalence of both disease entities. Available studies suggest that periodontitis prevalence in older subjects is high *Terpenning et al.*, *Persson et al.*, *HolmPedersen et al.*, *Krustrup & Petersen*. The aspect of aging as factor in the link between periodontitis and cardiovascular diseases including stroke must be considered in future studies.

The research on serum markers of inflammation in both cardiovascular and periodontal research is extensive. The literature clearly demonstrates that elevated proinflammatory cytokines are present in both cardiovascular diseases as well as in periodontitis. It appears that IL-6, PA-I, and WBC counts are closely related to periodontitis whereas the levels of serum CRP are not conclusive, *Persson & Persson et al.*,

Limitations of the study

Limitations of our study are that it includes wide age range of patients and also grading of periodontal disease severity was not done. To overcome this discrete age range of patients along with large sample size and future prospective interventional studies and randomized controlled clinical trials are suggested.

CONCLUSIONS

This observational study showed that there is a pathophysiological mechanism that links the cardiovascular diseases with periodontitis, serum C-reactive protein showed significant difference when compared between the gingivitis and periodontitis patients. Hence to conclude that human serum C-reactive protein may be used as cardiac biomarker in periodontitis patients. But further long-term studies with larger sample size are required to validate our results.

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