

Association of P.Gingivalis and CRP levels - A Case Control Study

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Abstract: This study was to evaluate the association of *P.gingivalis* with CRP levels in chronic generalized periodontitis subjects and periodontally healthy subjects. A total of 100 subjects aged between 20 yrs to 65 yrs were selected. Data was collected by an interview with the help of a proforma prepared for this study and dental examination was carried out. Subgingival plaque and blood samples were collected and were subjected for biochemical analysis. Results have shown that patients with the presence of *P.gingivalis* in the case group exhibited significantly elevated CRP level when compared to control group. They concluded that a significant relationship was observed between increased serum CRP level and the presence of *P.gingivalis*.

Keywords: C-reactive protein, *P.gingivalis*, Periodontal disease

I. Introduction

Destructive periodontal diseases are chronic inflammatory conditions characterized by connective tissue and alveolar bone destruction, eventually leading to tooth loss. Periodontal pathogens affect local and systemic immune and inflammatory responses. The local inflammatory response to these gram negative bacteria or bacterial products is characterized by infiltration of the periodontal tissues of inflammatory cells including polymorphonuclear neutrophils, macrophages, lymphocytes, and plasma cells [1]. Activated macrophages release cytokines, and some individuals respond to microbial challenge with an abnormally high delivery of such inflammatory mediators as PGE₂, IL-1 and TNF- α . These cytokines are involved in destruction of both periodontal connective tissue and alveolar bone. They can also initiate a systemic acute-phase response [2].

The host responds to the periodontal infections with an array of events involving both innate and adaptive immunity. Although periodontitis is chronic in nature, acute-phase elements are also part of the innate immunity in periodontitis and confirm that in periodontitis a systemic inflammation is present. The acute-phase reactants have pro-inflammatory properties; they activate complement factors, neutralize invasive pathogens and stimulate repair and regeneration of a variety of tissues. The acute-phase reactants receiving the most attention are C-reactive protein (CRP), plasminogen-activator 1 (PAI-1), and fibrinogen [3].

CRP is an acute phase protein which reflects a measure of the acute phase response. The term "acute phase" refers to local and systemic events that accompany inflammatory local response which includes vasodilatation, platelet aggregation, neutrophil chemotaxis, and release of lysosomal enzymes. Systemic responses include fever, leukocytosis, and a change in the hepatic synthesis of acute phase proteins. An acute phase protein has been defined as the one whose plasma concentration increases (positive acute phase proteins) or decreases (negative acute phase proteins) by at least 25% during inflammatory disorders. Recent evidence suggests the presence of chronic inflammatory periodontal disease may significantly affect systemic health conditions such as coronary heart disease (CHD), stroke, or adverse pregnancy outcomes [3].

Consequently, the relationship between periodontal disease and systemic disease (periodontal medicine) is a two-way road, with systemic host factors acting locally to reduce resistance to periodontal destruction and the local bacterial challenge generating widespread effects with the potential to induce adverse systemic outcomes. CRP is used as one of the markers of choice in monitoring the acute phase response because the markers increase to a relatively high concentration compared to basal concentration. Serial CRP measurement can be used as a diagnostic tool for finding clinical infections, monitoring effects of treatment outcome, and early detection of relapse of the disease, and hence can be a useful diagnostic aid in determining disease progression [3]. In this study an attempt has been made to evaluate the association of *P.gingivalis* with CRP levels in chronic generalized periodontitis subjects and periodontally healthy subjects.

II. Materials and methods

A 100 subjects were selected from the outpatient Department of Periodontology, Yenepoya dental college, Mangalore who gave consent to participate in the study. The subjects were grouped as Case Group (50 subjects with chronic generalized periodontitis), Control Group (50 subjects with healthy periodontium).

Inclusion criteria were age group 20-65 years, presence of a minimum of 20 permanent teeth except third molars, Case Group: Subjects with >30% sites with probing depth \geq 4mm, loss of attachment \geq 1mm, Control Group: Subjects with probing depth \leq 3mm and no loss of attachment. Patients were excluded if there was any known systemic disease, regular use of medication, presence of infection, use of antibiotics, non-steroidal anti-inflammatory drugs for last 3 months, pregnant or lactating females, any unhealed extraction socket and any traumatized tooth causing inflammation.

All clinical examinations were performed by one examiner. The parameters were recorded using community periodontal index. Probing and measuring were performed using WHO probe.

Plaque Sampling: Subgingival plaque samples were obtained with sterile paper points that are inserted into the sites of each subject and kept for one minute. Then plaque samples were suspended in a vial containing 0.5ml sterile double distilled water and carried to the laboratory. Detection of P.gingivalis was done by using polymerase chain reaction (PCR)

C-reactive protein measurement: The serum CRP levels were measured using latex turbidimetry. 2ml of non-fasting venous blood samples were collected from participants at the time of clinical examination. After collection of blood it was centrifuged for 5min at speed of 1000 rpm. After centrifugation 5 μ l sample (top layer) were taken into new test tube containing 450 μ l reagent 1 (Diluent) and 50 μ l reagent 2 (latex). Then the sample was placed into the semiautoanalyser (MISPA/Plus) and result was obtained within 2 min.

III. statistical analysis

The results obtained were recorded in a data sheet and was subjected to statistical analysis. Collected data was analyzed using Chi-square test, Mann Whitney test Z and Receiver operating curve.

IV. Results

The number of subjects in the case group was 50 with a mean CRP level of 4.13 ± 3.42 . The number of subjects in the control group was 50 with a mean CRP level of 1.26 ± 0.97 . The Mann Whitney test Z value for CRP level in the case and control group were 6.316 and the p value was 0.000 which is statistically highly significant (Table I). P.gingivalis was found to be positive in 40 subjects in the case group. In controls group P.gingivalis was found to be negative in all subjects. The Chi-square test value was 66.667. The p value was found to be 0.000 which is statistically highly significant (Table II). In the case group when CRP was compared with P.gingivalis using Mann Whitney test Z value showed 1.866 and the p value was 0.031 which is statistically significant (Table III). In control group P.gingivalis was not detected. Receiver operating curve analysis was done to obtain a cut off estimation for CRP levels which is > 1.65 mg/l and area under the curve showed 0.866 which is statistically significant.

V. Discussion

The purpose of this study was to evaluate the association of P.gingivalis with CRP levels in periodontally healthy and chronic periodontitis subjects. The results obtained in this study showed that P.gingivalis, CRP levels in the case group showed higher values when compared to control group. In this study, mean CRP levels were significantly higher in subjects with generalized periodontitis (4.13 mg/l) than in the controls (1.26 mg/l). These results are in accordance with the study by [4] where they observed that the mean CRP level in aggressive and rapid periodontitis patients (9 mg/l) and the controls were (2 mg/l), respectively. Similar results were also found in the study by Slade et al. where they reported a mean CRP value of 4.5 mg/l in subjects with $> 10\%$ of sites with PD ≥ 4 mm, 3.4 mg/l in subjects with less extensive periodontal pocketing, and 3.3 mg/l in periodontally healthy subjects[5]. These findings indicate a dose-response relationship between the extent of periodontitis and CRP.

Clinical signs of periodontal disease are a result of both bacterial infection and host response. Measures of infection, including quantifications of subgingival microorganisms and antibody responses to these pathogens, were suggested as a more direct measure of periodontitis for the studies of periodontal- systemic diseases association [6]. Antibodies to P. gingivalis were consistently associated with increased CRP levels among a representative sample of US adults [7], haemodialysis patients [8] and diabetics [9]. Two studies also reported higher CRP levels in subjects with at least one species of subgingival periodontal pathogen as compared with pathogen-negative individuals [2, 10]. Our results indicate that the presence of P. gingivalis in subgingival plaque, a probable indicator of current periodontal infection, was associated with periodontitis as well as increased CRP levels (p value 0.031). These results are in accordance with the study by Renuka DR et al where they found strong relationship between CRP level and presence of subgingival periodontal pathogen [11]. Pitiphat W et al. found that periodontitis and subgingival P.gingivalis are associated with increased CRP levels [12].

P.gingivalis is only one of several microorganisms having a possible role in the association between periodontitis and CRP. There are also several subclassifications of P.gingivalis, which may vary in the level of virulence. However, the PCR method used to detect P.gingivalis in this study is unable to neither quantify the number nor identify subclassification of the bacteria. Further studies are therefore needed to confirm the association of CRP and periodontitis with other microorganisms, to evaluate whether the association between CRP and P.gingivalis differs among different subclassifications, and to determine whether increased CRP levels are correlated with increased number of P.gingivalis. Elevation of CRP >3 mg/l is an indicator of high risk for CVDs in adults [13]. In our study, CRP level >1.6 mg/l can be considered as a risk indicator for disease state.

This study has certain limitations. Increase in CRP levels is non-specific. CRP is an indicator of a wide range of disease process including trauma, infections, and inflammation. To minimize these variations, we included in the study only apparently healthy individuals and excluded the participants if they had a history of infection, fever, trauma, or had taken medications within the recent past. Only three subjects (in the chronic periodontitis group) in our study had CRP greater than 10 mg/l, which indicated a threshold level of acute-phase effect [14], also suggesting the possibility of increased CRP by acute infection or trauma[12]. Another limitation in this study is that fasting blood samples, body mass index and cholesterol levels were not taken. Further studies are required for additional clinical parameters and also determine the effects of periodontal therapy on systemic inflammatory mediators.

Tables

Table I: CRP level among the groups

| CRP Level | N | Mean | Std. Deviation | 95% Confidence Interval for Mean | | Mannwhitney test Z value | P value |
|-----------|-----|------|----------------|----------------------------------|-------------|--------------------------|---------|
| | | | | Lower Bound | Upper Bound | | |
| CGP | 50 | 4.13 | 3.42 | 3.16 | 5.11 | 6.316 | 0.000 |
| Control | 50 | 1.26 | 0.97 | 0.99 | 1.54 | | |
| Total | 100 | 2.69 | 2.89 | 2.12 | 3.27 | | |

Table II: Presence of P.gingivalis among the groups

| | | Group | | Total |
|-------|----------|--------------|--------------|---------------|
| | | CGP | Control | |
| P.g | Negative | 10 20.0% | 50 100.0% | 60 60.0% |
| | Positive | 40 80.0% | 0 .0% | 40 40.0% |
| Total | | 50 100.0% | 50 100.0% | 100 100.0% |

Table III: Comparison of CRP level with P.gingivalis within the case group

| Group | P.g | N | Mean | Std. Deviation | 95% Confidence Interval for Mean | | Mannwhitney test Z value | P value |
|-------|----------|----|------|----------------|----------------------------------|-------------|--------------------------|---------|
| | | | | | Lower Bound | Upper Bound | | |
| CGP | Negative | 10 | 2.44 | 0.86 | 1.83 | 3.05 | 1.866 | 0.031 |
| | Positive | 40 | 4.56 | 3.69 | 3.37 | 5.74 | | |
| | Total | 50 | 4.13 | 3.42 | 3.16 | 5.11 | | |

VI. Conclusion

The following conclusions were drawn from the study:

1. Qualitative analysis revealed the presence of P.gingivalis in chronic generalized periodontitis subjects.
2. Semi-quantitative analysis revealed that serum CRP level was found to be increased in chronic generalized periodontitis subjects.

3. A significant relationship was observed between increased serum CRP level and the presence of *P.gingivalis*.

It is appropriate to conclude that periodontal infection may contribute to systemic inflammation.

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