Based on the periodontal status, 45 subjects were selected and divided into three groups. Group I - subjects with clinically healthy periodontium, Group II - generalized aggressive periodontitis (GAP), and Group III - chronic periodontitis (CP). Blood samples were collected from subjects for measurement of CRP. Periodontal parameters include plaque index (PI), gingival index, bleeding index (BI), probing pocket depth (PPD), and clinical attachment loss (CAL) were assessed. CRP levels were assessed by means of a commercially available high sensitivity-CRP enzyme immunoassay kit. Results: CRP levels were increased in Group III (6.0671 ± 3.15639 mg/L) and Group II subjects (4.5453 ± 2.88116 mg/L) compared to the Group I (1.0180 ± 0.94069 mg/L). CRP levels showed a positive correlation with all clinical parameters in Group I subjects. BI (r = 0.073), PI (r = 0.120) showed a positive correlation with CRP level in Group II and a positive correlation was also seen for PI (r = 0.492), PPD (r = 0.340), CAL (r = 0.160), and CRP level in Group III subjects. Conclusion: The mean CRP levels were found to be greater in CP compared to GAP subjects, but there was no statistically significant difference.

Keywords: Aggressive periodontitis, Chronic periodontitis, C-reactive protein

Introduction

Several reports have suggested that longstanding nature of periodontal disease results in the development of atherosclerosis, cardiovascular disease (CVD), cardiovascular accident, preterm low birth weight infants,[1,2] and changes in the lipid profile.[3] Risk factors for atherosclerosis include many factors, which also include subgingival Gram-negative pathogens as their presence on the atherosclerotic heart valves.[4,5] They release cytokines, involved in the destruction of periodontal tissues,[6] initiation of systemic acute phase response, and release of acute phase reactants such as C-reactive protein (CRP), fibrinogen, and serum amyloid A.[7]

CRP is a pentameric molecule and has several properties which include antibacterial, upregulation of proinflammatory cytokine production, a decrease in the development of autoimmune diseases, stimulation of repair, regeneration of a variety of tissues, and foam cell formation in atheromas.[8,9] In healthy individuals, CRP levels are found in trace amounts, i.e., <0.3 mg/l. Subjects with concentrations more than 3 mg/l in serum CRP are considered to be at higher a risk for future cardiovascular diseases and events.[10-12] After controlling for established risk factors which contribute to the elevation of CRP levels, increased levels of CRP persisted among individuals with the extensive periodontal disease.[13,14]

Chronic periodontitis (CP) is slow, and Aggressive periodontitis is rapid in disease progression. The estimation of CRP levels in periodontitis subjects may give the indication of an underlying pathway in the association between periodontal disease, CVD,[15] and impaired blood glucose levels.[16]

Materials and Methods

Forty-five (25 male and 20 female) subjects aged between 25 and 50 years, with mean age of 28.9 ± 1.2 and those who have not received any antibiotic therapy and periodontal therapy in the last 3 months with a minimum number of 20 teeth in the
oral cavity, with probing depth of ≥5 mm and with clinical attachment loss (CAL) ≥2, with good systemic health were included for the study from Department of Periodontics, Government Dental College, Hyderabad, India after getting the ethical clearance from the institute. Subjects were excluded if they had history of known systemic diseases and presence of other chronic infections, smokers and alcoholics, pregnant or lactating females, treatment with any medication known to affect the serum CRP levels, such as antibiotics, nonsteroidal anti-inflammatory drugs, oral contraceptive pills, 3-hydroxy-3-methylglutaryl-coenzyme A inhibitors in the past 3 months.

Based on periodontal status, subjects were divided into three groups by computer generated randomization. Group I (subjects with healthy periodontium), Group II generalized aggressive periodontitis (GAP), and Group III chronic generalized periodontitis. At baseline, blood samples were collected from subjects for measurement of CR P value. Periodontal parameters recorded included, plaque index (PI), gingival index (GI), bleeding index (BI), probing pocket depth (PPD) (distance from gingival margin to the base of the sulcus), CAL (distance from cementoenamel junction to the base of the pocket). For each subject, the periodontal disease status was evaluated at four sites per tooth by two examiners (Buccal, Distobuccal, Mesiobuccal, and lingual) measured using UNC 15 probe (Hu-Friedy’s, USA).

Ten milliliter of blood sample was collected from the brachial vein from each of the subjects, by aseptic technique using a 10cc syringe, transferred to an appropriately labeled tube and centrifuged for 15 min at 3000 RPM separating the cells from the serum and the smear layer was removed carefully.

Separated serum was collected in eppendorf and stored in the deep freeze at −20°C. Serum CRP levels were assessed by means of a commercially available high sensitivity-CRP Enzyme Immunoassay* (The EiAsy™Way, Diagnostics Biochem, Canada Inc., Canada).

Mean values of each parameter were compared between the groups using one-way analysis of variance with post hoc test of least significance difference method. Relationships between the parameters were assessed by Pearson’s correlation coefficient. Analysis of covariance was used for comparison of mean values between the groups to adjust the ages. The test values were considered statistically significant at P < 0.05.

Results

The mean age of the subjects in Groups I, II, and III were 31.13 ± 8.55 years, 29.27 ± 3.77 years, and 36.73 ± 7.38 years, respectively. This difference was found to be statistically not significant (P = 0.27). The mean PI scores in the Groups I, II, and III were 0.69 ± 0.17, 1.63 ± 0.57, and 1.71 ± 0.30, respectively. The mean BI scores in the Groups I, II, and III were 36.10 ± 15.97, 94.96 ± 10.48, and 93.10 ± 6.79, respectively. The mean GI scores in the Groups I, II, and III were 0.46 ± 0.21, 2.07 ± 0.52, and 2.1 ± 0.52, respectively. The mean CAL scores in the Groups I, II, and III were 1.30 ± 0.31 mm, 4.34 ± 0.64 mm, and 4.37 ± 0.75 mm, respectively [Table 1].

The mean CRP concentration in the Groups I, II, and III was 1.01 ± 0.94 mg/L, 4.54 ± 2.88 mg/L, and 6.06 ± 3.15 mg/L, respectively. Intergroup comparison showed statistically significant difference (P = 0.012) in the CRP level between Groups I and II, and between Groups II and III [Figure 1].

A statistically significant difference was found in the PPD and CRP scores of Groups I and II, Groups I and III, and Groups II and III. CRP levels did not show a positive correlation with all clinical parameters in Group I subjects. BI (r = 0.073), PI (r = 0.120) showed a positive correlation with CRP level in group II and a positive correlation was also seen for PI (r = 0.492), PPD (r = 0.340), CAL (r = 0.160) and CRP level in Group III subjects. The difference in the mean scores of PI, BI, GI, and CAL between Groups I and II, and Groups I and III was found to be statistically significant (P < 0.05), whereas the difference in the BI scores of Group II and III was not found to be statistically significant (P = 0.25) [Table 2].

Discussion

The present study showed an increase in serum CRP levels in CP and GAP subjects compared to the controls. This is in accordance with the results of earlier studies which have shown an elevation in CRP levels in periodontitis patients.[10,13,19,20]

In this study, more than 50% of subjects with CP and GAP demonstrated a mean CRP level >3 mg/L, the level of CRP concentration reported to be at risk for development of cardiovascular disease. The findings of the present study are in agreement with similar studies by Slade et al.[13] and Wu et al.[21] showed a significant correlation between periodontal status and prevalence of elevated CRP. However, these studies used the NHANES III database and CRP levels <3 mg/L were not measured in these studies.

Table 1: Comparison of periodontal parameters among the groups

|                  | Group I     | Group II    | Group III   |
|------------------|-------------|-------------|-------------|
| PI               | 0.695±0.1704| 1.637±0.574 | 1.711±0.306 |
| GI               | 0.466±0.212 | 2.072±0.528 | 2.108±0.528 |
| BI               | 36.109±15.971| 94.961±10.480| 93.673±6.796|
| PPD              | 2.046±0.555 | 5.598±0.834 | 4.424±0.410 |
| CAL              | 1.301±0.311 | 4.346±0.642 | 4.376±0.759 |

*pOne-way analysis using post hoc test. CAL: Clinical attachment loss; PPD: Probing pocket depth; BI: Bleeding index; GI: Gingival index; PI: Plaque index; SD: Standard deviation
Fredriksson et al., Ebersole et al., Koppolu et al. found higher serum CRP concentrations in periodontitis patients than in controls. While Ebersole et al. observed a relationship between CRP levels and the absence or presence, or severity of, adult periodontitis, it is not evident whether the study design controlled for the effects of smoking, a known factor for both serum markers concentration and periodontitis. The later study reported about 9 mg/L, roughly one and half times that of the CRP concentrations of the present study. This difference could be caused by the investigation of more severe periodontitis cases in their study. Furthermore, their study did not adjust for potential confounders such as age, smoking, body mass index (BMI), and blood lipids in particular, since hyperlipidemia has been associated with periodontal disease.

Very few studies have evaluated CRP levels in aggressive periodontitis subjects. One study by Salzberg et al. reported an increase in CRP levels in GAP subjects (3.72 mg/L) and the observations are similar to the findings of the present study (4.54 mg/L). In the present study, though the CRP levels were found to be elevated in aggressive periodontitis group of subjects, the mean CRP levels were found to be lower when compare to CP group. The reason for this difference in CRP levels between aggressive and CP groups is not exactly understood at this point of time, but could be attributable to the long standing nature and chronic course of the disease process in CP, thus exerting its systemic influence over a longer period compared to aggressive periodontitis which runs a shorter course.

In addition to chronic infections, CRP serum concentrations are reported to be positively correlated with age, smoking, BMI, or lipid parameters. Since the mean age among the groups differed significantly in the present study, with the lowest being in the control group followed by aggressive periodontitis group and CP group, age was included as a potential confounding parameter in covariance analysis. Such an analysis showed that the age did not influence the mean CRP levels of the study.

In the present study, clinical parameters such as bleeding on probing (BOP) showed a positive correlation with CRP level in aggressive periodontitis group and a positive correlation was also seen for PPD, clinical attachment level, and CRP in CP group of subjects. This is in accordance with the earlier studies which reported that increased BOP, probing depth and attachment loss to be significantly associated with elevated CRP concentrations.

Glurich et al. found significantly elevated CRP levels in subjects with BOP and mean CAL ≥4 mm. Noack et al. found a significant increase of serum CRP in subjects with 3 mm mean CAL. Slade et al. reported a mean CR P value of 4.5 mg/L in subjects with ≥10% of siteS with PPD ≥4 mm. These findings indicate a dose – response relationship between the extent of periodontitis and CRP. The results of the present study corroborate the observations of the previous studies indicating that periodontal diseases are associated with elevated serum CRP levels. Elevation of CRP such as that seen in periodontal diseases may supplement systemic vascular inflammation, atheroma formation and add to the preexisting risk for cardiovascular sequelae. If periodontitis can lead to the elevation of CRP levels, then theoretically, periodontal therapy should help in reducing the systemic burden of inflammation. However, to know whether such benefit can really be translated in the long-term can only be assessed in future by well-controlled longitudinal clinical trials. Further studies should focus on the relationship between periodontitis, elevated CRP levels and the effect of periodontal therapy on serum CRP concentration. The results of the present study indicated an increase in serum CRP levels in subjects with GAP and CP compared to controls. The CRP levels in CP subjects were higher when compared to subjects with aggressive periodontitis, but this was not found to be statistically significant. However, the results of the present study cannot be used to determine the causality of the associations between periodontitis and CRP due to some limitations, one being the small sample size and the

**Table 2: Correlation between C-reactive protein levels with parameters**

| Correlation co-efficient (r) of CRP | Group I (r) | Group II (r) | Group III (r) |
|------------------------------------|------------|--------------|---------------|
| CRP versus age                     | -0.060     | 0.831        | 0.453         |
| CRP versus PI                       | 0.062      | 0.827        | 0.120         |
| CRP versus BI                       | 0.134      | 0.635        | 0.073         |
| CRP versus GL                       | 0.061      | 0.942        | -0.125        |
| CRP versus PPD                      | 0.081      | 0.775        | -0.297        |
| CRP versus CAL                      | 0.348      | 0.204        | -0.306        |

Pearson correlation test. CAL: Clinical attachment loss; PPD: Probing pocket depth; BI: Bleeding index; GI: Gingival index; PI: Plaque index; CRP: C-reactive protein
other is that the study is only cross-sectional. Moreover, the subjects might have undiagnosed systemic factors and other undetected risk factors that could influence the CRP levels. It would be appropriate if large sample based, well controlled, longitudinal trials are performed to determine the relationship between periodontitis and elevated CRP levels and effect of periodontal therapy on serum CRP concentration.

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Conflicts of interest
There are no conflicts of interest.

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