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**Research Article**

# Evaluation of Serum C reactive protein levels in Periodontitis Patients and Healthy Subjects

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**Abstract:** **Background and Objectives**: CRP is currently regarded as a biomarker of systemic inflammation. Several studies have examined the relationship between periodontitis and CRP using various designs including observational, cross-sectional (case– control) and longitudinal studies. Most studies to date have evaluated C-reactive proteins levels in patients with chronic periodontitis but few have investigated C-reactive protein levels in subjects with aggressive periodontitis. The purpose of this study is to determine the levels of serum C-reactive proteins in aggressive and chronic generalized periodontitis and to assess if their levels vary among the two types of periodontitis in comparison to healthy subjects.**Methods**: A total of 90 systemically healthy patients in the age group of 25-50 years were taken up for the study. Based on probing pocket depth (PPD) and clinical attachment loss (CAL), they were allotted equally into 3 groups: Generalized Aggressive Periodontitis (Group A); Chronic Generalized Periodontitis (Group B); Healthy controls (Group C). Clinical examinations together with quantitative determination of CRP was done using highly sensitive Immunoturbidometric method on the 3 groups A, B, C **Results**: The CRP levels were greater in generalized aggressive periodontitis group than those in chronic generalized periodontitis group, which in turn were greater than the controls. Multiple comparisons made among the three groups showed no statistically significant difference between groups although the absolute values were higher in the patient groups.**Conclusion:** CRP is a non-specific marker of the acute-phase response. That is, many potential stimuli, including (unknown) chronic infections and or inflammatory conditions, smoking, obesity and trauma, may also account for mild increases in CRP**.**

**Keywords:** C-reactive protein; periodontal destruction; attachment loss; Immunoturbidometry; aggressive periodontitis.

## INTRODUCTION

The pathological role of the subgingival micro biota in the initiation and progression of periodontitis is well accepted. Periodontal pathogen affects local and systemic immune and inflammatory responses ( Socransky, S. S., *et al.*,1998). The local inflammatory response to these bacteria or bacterial products is characterized by infiltration of the periodontal tissue by inflammatory cells including polymorphonuclear neutrophils (PMNLs), macrophages, lymphocytes and plasma cells. Activated macrophages release cytokines and some individuals respond to microbial challenge with an abnormally high delivery of such inflammatory mediators as Prostaglandins $E_2$ ($PGE_2$), Interleukins-1 (IL-1), and Tumor Necrosis Factor-ά (TNF- ά). These cytokines are involved in the destruction of both the periodontal connective tissue and alveolar bone.( Ebersole, J. L., & Cappelli, D. 2000)

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.( Ebersole, J. L., & Cappelli, D. 2000)

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

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C-reactive protein (CRP), plasminogen-activator 1 (PAI-1), and fibrinogen. CRP in particular has been the focus of attention as a key marker of atherosclerosis and elevated levels (e.g. ≥2.1 mg/l) constitute a risk predictor for cardiovascular disease (CVD). ( Danesh, J.,*et al*.,1998; Blake, G. J., & Ridker, P. M. 2001; Blake, G. J., & Ridker, P. M. 2001; Blake, G. J., & Ridker, P. M. 2002; Blake, G. J.,*et al*.,2003)

CRP rises in serum or plasma within 24-48 hours following acute tissue damage, reach a peak during the acute stage (as high as thousand fold) and decreased with the resolution of inflammation or trauma. In healthy individuals, CRP levels are found in trace amounts with levels of <0.3 mg/l. Serum levels of CRP could exceed 100 mg/l in the presence of overwhelming systemic infection, which provides a useful marker for tracking the course of infection.

Importantly, CRP is currently regarded as a biomarker of systemic inflammation. Several studies have examined the relationship between periodontitis and CRP using various designs including observational, cross-sectional (case-control) and longitudinal studies. Most studies to date have evaluated C-reactive proteins levels in patients with chronic periodontitis but few have investigated C-reactive protein levels in subjects with aggressive periodontitis.( Salzberg, T. N.,*et al*.,2006)

The purpose of this study is to determine the levels of serum C-reactive proteins in aggressive and chronic generalized periodontitis and to assess if their levels vary among the two types of periodontitis in comparison to healthy subjects.

## MATERIALS & METHODS

Following complete medical and dental examination, 90 systemically healthy individuals attending the Department of Periodontics, M S Ramaiah Dental College, Bengaluru were selected for the study. The study was approved by the institutional ethics committee. Based on probing pocket depth (PPD) and clinical attachment loss (CAL) values, a total of 90 subjects were equally allotted to one of the 3 groups: Group A, generalized aggressive periodontitis (GAP) patients; Group B, chronic generalized periodontitis (CGP) patients and Group C, healthy individuals. Thus, each group consisted of 30 subjects.

## INCLUSION CRITERIA

**Subjects Were Divided Into 3 Groups Based On Following Criteria:**

- Group A – GAP: Patients under age of 30 years; PPD of ≥ 5mm and/or CAL involving first molars and incisors and at least 3 other permanent teeth; local factors being inconsistent with disease severity.
- Group B – CGP: PPD of ≥ 5 mm and/or CAL at ≥ 30 percent of the teeth present; local factors concomitant with amount of periodontal destruction and with moderate rate of progression.
- Group C – Healthy controls: Clinically healthy periodontal status with PPD ≤ 2 mm and no evidence of attachment loss and no history of systemic disease.

## EXCLUSION CRITERIA

- Patients having systemic disorders that may affect the study outcome like diabetes mellitus, rheumatoid arthritis etc.
- Pregnant and lactating patients.
- Patients who have taken antibiotics in the past 6 months.
- Patients who have undergone any periodontal therapy in the past 6 months.
- Tobacco users

## PERIODONTAL ASSESSMENT

All patients taking part in the study signed an informed consent form. A complete case history was recorded in a specially prepared proforma and radiographs were taken to assess the amount of bone loss. The following clinical parameters were recorded for all the study subjects.

- Plaque Index (Sillness & Loe,1964)
- Gingival Index (Loe & Silliness,1963)
- Probing pocket depth (PPD)
- Clinical attachment loss (CAL)
- Bleeding Index.(Muhlemann and Son, 1971)
- Presence or absence of suppuration.

All the clinical examinations were performed at 4 sites per tooth (mesiobuccal, buccal, distobuccal and lingual/palatal) using a periodontal probe.* The PPD was measured as the distance from the gingival margin to the base of the pocket in millimeter. The CAL values were expressed as distance from the cementoenamel junction to the base of the pocket in millimeters. Quantitative determination of CRP was done using highly sensitive immunoturbidometric method on the 3 groups A, B, C.*(* Otsuji, S.,*et al*.,1982*)*

## SAMPLE COLLECTION

Venous blood was withdrawn from the participants selected for the study. They were made to tighten a fist so that vein was more palpable, and antecubital vein was selected for venipuncture. A tourniquet was applied about 1-2 inches above the antecubital fossa. After cleansing the puncture site with 10% isopropanol solution, blood was withdrawn using a syringe with 24 gauge needle. Tourniquet was released as the blood flow began. After drawing 3 ml of blood, sterile cotton ball was placed on the puncture site and needle was withdrawn. The subjects were instructed to apply mild finger pressure on the site for few minutes to avoid oozing out of blood (Figure 1).

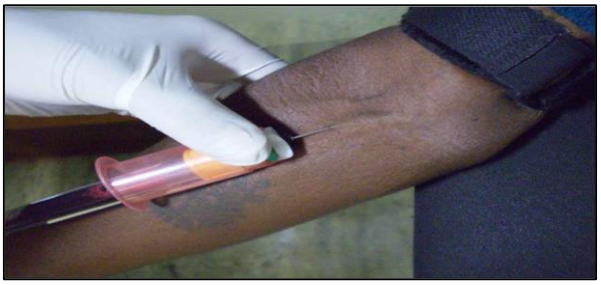

**Figure 1: Collection of blood sample**

## CRP DETERMINATION

The collected blood samples were centrifuged in a centrifugal machine at 3000 rpm for 10 minutes to separate the serum from blood (Figure 2).

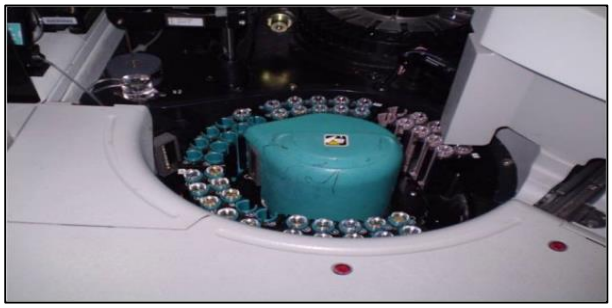

**Figure 2: Centrifuge used for preparation of serum**

Serum levels of CRP were quantified using a high sensitivity CRP enzyme-linked immunosorbent assay (hsCRP ELISA). A reagent† was used to quantify the levels of CRP (Figure 3).


**Figure 3: Reagents used for estimation of serum CRP levels**

Lower limits of hsCRP ELISA were 1 mg/l CRP, and the upper limits were 150 mg/l CRP. The serum CRP levels for each subject were exhibited digitally on a computer screen (Figure 4) which were printed out and stored for statistical analysis.


**Figure 4: Digital read-out of serum CRP levels in blood sample**

## PRINCIPLE OF THE ASSAY

The CRP in a sample reacts with the specific antibody producing insoluble immune complexes. The turbidity caused by these immune complexes is proportional to the CRP concentration in sample and can be measured spectrophotometrically.

## STATISTICAL ANALYSIS

Analysis of variance has been used to find the significance of periodontal parameters and CRP mg/L between the three groups and post-hoc Tukey test has been used to find the pair wise significance between the groups. Pearson correlation was used to find the relationship of changes in periodontal parameters with the changes in CRP levels for both Group A and Group B. All levels of significance were set at $P < 0.05$.

## RESULTS

The age of the patients ranged between 20 to 50 years. The mean age of patients in group A, B, and C were 31.63 ± 6.36 yrs, 35.97 ± 8.21 yrs and 27.10 ± 2.88 yrs. The percentage of males in each group was 56.7%, 50.0% and 73.3% and that of females were 43.3 %, 50.0 % and 26.7 % respectively (Table 1).

**Table 1: Subject characteristics of 3 groups**

| Characteristic | Group A (n=30) | Group B (n=30) | Group C (n=30) | P value |
|---|---|---|---|---|
| Males | 17 | 15 | 22 | >0.164 |
| Females | 13 | 15 | 8 | |
| | Mean±SD | Mean±SD | Mean±SD | |
| Age | 31.63±6.3 | 35.97±8.2 | 27.1±2.8 | <0.01* |
| Number of teeth | 28.4±3.5 | 29.73±3.1 | 30.43±1.9 | >0.029 |

All groups were different with respect to clinical characteristics, with generally more severe clinical indices in group B (CGP) and less severe in group A (GAP) and control group. The mean probing depth and attachment loss of group A, B and C were 5.6257, 4.9203, 1.8507 and 5.6777, 5.2047, 0 respectively which was statistically significant among the groups (Table 2).

**Table 2: Comparison of clinical parameters among 3 groups**

| Clinical Parameter | Group A (n=30) | Group B (n=30) | Group C (n=30) | P value |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| PPD | 5.6±0.5 | 4.9±0.5 | 1.8±0.4 | <0.01* |
| CAL | 5.6±0.4 | 5.2±0.6 | 0.0±0.0 | < 0.02* |
| PI | 1.2±0.4 | 1.3±0.2 | 0.7±0.1 | <0.01* |
| GI | 1.1±0.4 | 1.3±0.2 | 0.6±0.1 | <0.01* |
| BI | 1.8±0.3 | 1.9±0.3 | 0.7±0.1 | <0.01* |

The mean CRP levels for the three groups A, B and C were 4.36 mg/L, 3.62 mg/L and 2.1 mg/L respectively (Table 3).

**Table 3: Mean CRP levels in study groups**

| | Range(mg/l) | Mean(mg/l) | SE |
|---|---|---|---|
| Group A | 1.0 - 44.8 | 4.36 | 1.4456 |
| Group B | 1.0 – 13.7 | 3.62 | 0.6614 |
| Group C | 0.7 – 8.6 | 2.1 | 0.2891 |

As the CRP values were skewed, statistical analysis was performed on the log-transformed data. As shown in Table 4**,** the log CRP values of the three patient groups were not statistically different from each other ($p < 0.085$); however the CRP levels were greater in generalized aggressive periodontitis group greater than those in chronic generalized periodontitis group, which in turn were greater than the controls.

**Table 4: Concentration of Log CRP levels in study groups**

| Groups | CRP levels (Geometric mean±SD) | F | P value |
|---|---|---|---|
| Group A | 0.4438±0.32213 | 2.533 | 0.085 |
| Group B | 0.3759±0.33700 | | |
| Group C | 0.2696±0.23749 | | |

Finally multiple comparisons were made using Pearson correlation test among the three groups for CRP, which showed no statistically significant difference between groups although the absolute values were higher in the patient groups (Table 5).

**Table 5: Multiple comparisons of CRP between groups**

| (I) Group | (J) Group | Mean Difference (I-J) | Std. Error | P value | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Group A | Group B | 0.9523 | 1.3193 | 0.472 | -1.670 | 3.574 |
| | Group C | 2.1633 | 1.3193 | 0.105 | -.459 | 4.785 |
| Group B | Group A | -.9523 | 1.3193 | 0.472 | -3.574 | 1.670 |
| | Group C | 1.2110 | 1.3193 | 0.361 | -1.411 | 3.833 |
| Group C | Group A | -2.1633 | 1.3193 | 0.105 | -4.785 | .459 |
| | Group B | -1.2110 | 1.3193 | 0.361 | -3.833 | 1.411 |

* p value <0.05 indicates statistical significance

## DISCUSSION

A number of studies have demonstrated an association between periodontal disease and the risk of myocardial infarction and stroke as well as the underlying condition atherosclerosis.( Janket, S. J.,*et al*.,2003; Meurman, J. H.,*et al*.,2004; Desvarieux, M.,*et al*.,2005; Leivadaros, E.,*et al*.,2005; Söder, P. O.,*et al*.,2005) The association is believed to be either due to direct effects of periodontal pathogens or indirect activation of host mediated immunity. 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.( Ebersole, J. L., & Cappelli, D. 2000; Loos, B. G. 2005)

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. CRP in particular has been the focus of attention as a key marker of atherosclerosis and elevated levels constitute a risk predictor for cardiovascular disease (CVD).( Scannapieco, F. A. 1998)

We evaluated the levels of serum C-reactive proteins in aggressive and chronic generalized periodontitis and assessed if their levels vary among the two types of periodontitis in comparison to healthy subjects. The mean CRP for group A (GAP) was 4.36 mg/l whereas for group B (CGP) and group C (healthy controls), it was 3.62 mg/ml and 2.1 mg/l. As the CRP values were skewed, statistical analysis was performed on the log-transformed data which showed difference in

mean CRP levels among three groups were statistically not significant.

Earlier studies of acute-phase reactants in periodontitis have focused more on patients with chronic periodontitis and such studies have demonstrated that CRP levels are higher in periodontitis patients than in periodontally healthy subjects. The investigators also argued that the serum CRP levels are higher in patients with more severe form of periodontitis.( Ebersole, J. L.,*et al*.,1997; Loos, B. G.,*et al*.,2000; Noack, B.,*et al*.,2001; Craig, R. G.,*et al*.,2003; Buhlin, K.,*et al*.,2003; Saito, T.,*et al*.,2003; Persson, G. R.,*et al*.,2005) In our study, we found higher CRP levels in generalized aggressive periodontitis patients as compared to those in chronic periodontitis but the difference was statistically non-significant.

Except a few subjects, none of the patients in the present study had CRP levels > 10.0 mg/l indicating that it is relatively unlikely that the subjects were experiencing acute or chronic systemic diseases characterized by a large increase in serum CRP levels.( Pearson, T. A.,*et al*.,2003) Moreover, 10 patients of group A and 7 patients of group B showed values of ≥ 3 mg/ L respectively, while only 3 patients had values ≥ 3 mgs/ L in group C suggesting the possibility that severity of periodontal disease has a relatively higher risk of cardiovascular events.( Blake, G. J., & Ridker, P. M. 2001; Blake, G. J., & Ridker, P. M. 2001)

In addition, recent trials have indicated that treatment of periodontal infections, whether by intensive mechanical therapy, drug therapy, or extraction, can significantly lower serum levels of CRP. All such studies portray periodontal infection or its resultant inflammatory response as a source of systemic elevation of serum CRP.

## CONCLUSION

In the present study, we could not show convincing evidence that CRP is consistently elevated in periodontitis patients compared with healthy controls. It needs to be stressed that CRP is a non-specific marker of the acute-phase response. Many potential stimuli such as (unknown) chronic infections and or inflammatory conditions, smoking, obesity and trauma may also account for mild increases in CRP. It is therefore fair to speculate that periodontitis, in addition to other factors and acute/chronic infections will result in moderately elevated levels of CRP and perhaps in part via this acute-phase response reactant may contribute to a higher risk for CVD.( D'aiuto, F.,*et al*.,2005; Mattila, K.,*et al*.,2002; Yamazaki, K.,*et al*.,2005)

## REFERENCES


1. Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C., & Kent Jr, R. L. (1998). Microbial complexes in subgingival plaque. *Journal of clinical periodontology*, *25*(2), 134-144.
2. Ebersole, J. L., & Cappelli, D. (2000). Acute-phase reactants in infections and inflammatory diseases. *Periodontology 2000*, *23*(1), 19-49.
3. Loos, B. G. (2005). Systemic markers of inflammation in periodontitis. *Journal of periodontology*, *76*, 2106-2115.
4. Danesh, J., Collins, R., Appleby, P., & Peto, R. (1998). Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. *Jama*, *279*(18), 1477-1482.
5. Blake, G. J., & Ridker, P. M. (2001). High sensitivity C-reactive protein for predicting cardiovascular disease: an inflammatory hypothesis. *European heart journal*, *22*(5), 349-352.
6. Blake, G. J., & Ridker, P. M. (2003). C-reactive protein: a surrogate risk marker or mediator of atherothrombosis?. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *285*(5), R1250-R1252.
7. Blake, G. J., & Ridker, P. M. (2002). Inflammatory bio-markers and cardiovascular risk prediction. *Journal of internal medicine*, *252*(4), 283-294.
8. Blake, G. J., Rifai, N., Buring, J. E., & Ridker, P. M. (2003). Blood pressure, C-reactive protein, and risk of future cardiovascular events. *Circulation*, *108*(24), 2993-2999.
9. Salzberg, T. N., Overstreet, B. T., Rogers, J. D., Califano, J. V., Best, A. M., & Schenkein, H. A. (2006). C-reactive protein levels in patients with aggressive periodontitis. *Journal of periodontology*, *77*(6), 933-939.
10. Otsuji, S., Shibata, H., & Umeda, M. (1982). Turbidimetric immunoassay of serum C-reactive protein. *Clinical chemistry*, *28*(10), 2121-2124.
11. Janket, S. J., Baird, A. E., Chuang, S. K., & Jones, J. A. (2003). Meta-analysis of periodontal disease and risk of coronary heart disease and stroke. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, *95*(5), 559-569.
12. Meurman, J. H., Sanz, M., & Janket, S. J. (2004). Oral health, atherosclerosis, and cardiovascular disease. *Critical Reviews in Oral Biology & Medicine*, *15*(6), 403-413.
13. Desvarieux, M., Demmer, R. T., Rundek, T., Boden-Albala, B., Jacobs Jr, D. R., Sacco, R. L., & Papapanou, P. N. (2005). Periodontal microbiota and carotid intima-media thickness: the Oral Infections and Vascular Disease Epidemiology Study (INVEST). *Circulation*, *111*(5), 576-582.
14. Leivadaros, E., Van der Velden, U., Bizzarro, S., ten Heggeler, J. M., Gerdes, V. E., Hoek, F. J., ... & ten Cate, H. (2005). A pilot study into measurements of markers of atherosclerosis in periodontitis. *Journal of periodontology*, *76*(1), 121-128.

15. Söder, P. O., Söder, B., Nowak, J., & Jogestrand, T. (2005). Early carotid atherosclerosis in subjects with periodontal diseases. *Stroke*, *36*(6), 1195-1200.
16. Scannapieco, F. A. (1998). Periodontal disease as a potential risk factor for systemic diseases: position paper of The American Academy of Periodontolagy. *J Periodotol*, *69*, 841-850.
17. Ebersole, J. L., Machen, R. L., Steffen, M. J., & Willmann, D. E. (1997). Systemic acute-phase reactants, C-reactive protein and haptoglobin, in adult periodontitis. *Clinical & Experimental Immunology*, *107*(2), 347-352.
18. Loos, B. G., Craandijk, J., Hoek, F. J., Wertheim-van Dillen, P. M., & Van Der Velden, U. (2000). Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *Journal of periodontology*, *71*(10), 1528-1534.
19. Noack, B., Genco, R. J., Trevisan, M., Grossi, S., Zambon, J. J., & De Nardin, E. (2001). Periodontal infections contribute to elevated systemic C-reactive protein level. *Journal of periodontology*, *72*(9), 1221-1227.
20. Craig, R. G., Yip, J. K., So, M. K., Boylan, R. J., Socransky, S. S., & Haffajee, A. D. (2003). Relationship of destructive periodontal disease to the acute-phase response. *Journal of periodontology*, *74*(7), 1007-1016.
21. Buhlin, K., Gustafsson, A., Pockley, A. G., Frostegård, J., & Klinge, B. (2003). Risk factors for cardiovascular disease in patients with periodontitis. *European heart journal*, *24*(23), 2099-2107.
22. Saito, T., Murakami, M., Shimazaki, Y., Oobayashi, K., Matsumoto, S., & Koga, T. (2003). Association between alveolar bone loss and elevated serum C-reactive protein in Japanese men. *Journal of periodontology*, *74*(12), 1741-1746.
23. Persson, G. R., Pettersson, T., Ohlsson, O., & Renvert, S. (2005). High-sensitivity serum C-reactive protein levels in subjects with or without myocardial infarction or periodontitis. *Journal of clinical periodontology*, *32*(3), 219-224.
24. Pearson, T. A., Mensah, G. A., Alexander, R. W., Anderson, J. L., Cannon III, R. O., Criqui, M., ... & Rifai, N. (2003). Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *circulation*, *107*(3), 499-511.
25. D'aiuto, F., Nibali, L., Parkar, M., Suvan, J., & Tonetti, M. S. (2005). Short-term effects of intensive periodontal therapy on serum inflammatory markers and cholesterol. *Journal of dental research*, *84*(3), 269-273.
26. Mattila, K., Vesanen, M., Valtonen, V., Nieminen, M., Palosuo, T., Rasi, V., & Asikainen, S. (2002). Effect of treating periodontitis on C-reactive protein levels: a pilot study. *BMC infectious diseases*, *2*(1), 30.
27. Yamazaki, K., Honda, T., Oda, T., Ueki-Maruyama, K., Nakajima, T., Yoshie, H., & Seymour, G. J. (2005). Effect of periodontal treatment on the C-reactive protein and proinflammatory cytokine levels in Japanese periodontitis patients. *Journal of periodontal research*, *40*(1), 53-58.