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Elevation of C-reactive protein and interleukin-6 in plasma of patients with aggressive periodontitis

X. J. Sun, H. X. Meng, D. Shi, L. Xu, L. Zhang, Z. B. Chen, X. H. Feng, R. F. Lu, X. Y. Ren

Department of Periodontology, Peking University School and Hospital of Stomatology, Beijing, China

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Background and Objective: Systemic levels of C-reactive protein and interleukin-6 have been reported to be elevated in patients with periodontitis compared with periodontally healthy individuals. Most studies included patients with chronic periodontitis and comprised predominantly Caucasians. The aim of this study was to determine the relative levels of C-reactive protein and interleukin-6 in plasma of patients with aggressive periodontitis in China and to examine the relationships between these two inflammatory mediators and clinical parameters, peripheral blood cells and protein variables.

Material and Methods: Plasma samples were collected from 84 patients with aggressive periodontitis and from 65 control subjects. Periodontal examination consisted of taking probing depth and attachment loss measurements. The levels of plasma C-reactive protein and interleukin-6 were determined using enzyme-linked immunosorbent assays.

Results: The levels of plasma C-reactive protein in patients with aggressive periodontitis were significantly higher than those in control subjects (1.87 vs. 0.52 mg/L). The level of plasma interleukin-6 in patients with aggressive periodontitis was 1.20 pg/mL, higher than that in control subjects (0.08 pg/mL). Multiple linear regression analysis showed that log C-reactive protein was significantly related to severe sites percentage and albumin following correction for age, gender, body mass index and smoking ($p = 0.000$, $p = 0.008$, respectively). Log interleukin-6 was found to be significantly correlated with periodontal diagnosis, leukocyte count and level of fasting blood glucose after adjusting for the confounders ($p = 0.000$, $p = 0.009$ and $p = 0.013$, respectively).

Conclusion: Patients with aggressive periodontitis have significantly elevated levels of plasma C-reactive protein and interleukin-6. These elevated inflammatory factors might potentially increase the risk for cardiovascular events and glucose dysregulation in relatively young individuals.

Huan Xin Meng, Department of Periodontology, Peking University School and Hospital of Stomatology, 22 Zhongguancun Nandajie, Haidian District, 100081 Beijing, China
Tel: 0086 10 62179977 ext. 2522 (O)
Fax: 0086 10 62173402
e-mail: kqhxmeng@bjmu.edu.cn

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Several parameters of systemic inflammation have been identified to be associated with cardiovascular diseases and diabetes. For example, C-reactive protein has been shown to be a strong predictor of cardiovascular events, and the levels of C-reactive protein are increased in the serum of diabetic patients (1,2). Also, elevated levels of interleukin-6 in peripheral blood have been associated with unstable angina and metabolic dysregulation (3,4). Furthermore, periodontitis has been proposed to relate to the elevation of systemic levels of C-reactive protein and interleukin-6 (5–7). As periodontal infections may increase the risk of atherosclerosis and poor glycemic

control in diabetic patients (8,9), it is postulated that C-reactive protein and interleukin-6 might be possible mediators involved in the association between periodontitis and systemic diseases.

However, most studies of inflammatory mediators in periodontitis reported previously have focused on patients with chronic periodontitis (5–7), and only one study of C-reactive protein in aggressive periodontitis can be found (10). Aggressive periodontitis is a particular type of periodontal tissue inflammation, characterized by the rapid destruction of tooth-supporting tissues and leading to preterm dentition loss in young people. The aggressive nature and early onset of the disease have been found to depend on many factors, including host susceptibility. Our previous studies demonstrated that the plasma levels of triglycerides, fasting glucose and interleukin-1β of patients with aggressive periodontitis were all significantly higher than those of the controls (11,12). Therefore, the question arises of whether abnormalities in the plasma concentrations of C-reactive protein and interleukin-6 exist in patients with aggressive periodontitis and whether these abnormalities are responsible for the association of periodontitis with these systemic conditions, as well as systemic diseases. The aim of this study was to determine the relative plasma levels of C-reactive protein and interleukin-6 in the same cohort of patients with aggressive periodontitis reported on in our previous studies (11,12) and to analyze the relationships between these inflammatory mediators and clinical parameters, peripheral blood cells and protein variables.

Material and methods

Study population

Eighty-four subjects with generalized aggressive periodontitis were patients of the Department of Periodontology at Peking University School of Stomatology. The diagnostic criteria for aggressive periodontitis were defined according to the classification proposed at the International Workshop for the Classification of Periodontal Diseases and Conditions in 1999. The details were as follows.

(i) The onset of periodontal disease occurred when the subject was under 35 years of age.

(ii) The subject had to have least eight teeth with probing depth > 6 mm and radiographic evidence of alveolar bone loss, and at least three of these teeth were not to be first molars or incisors.

Sixty-five control subjects were recruited from the staff and students at the School of Stomatology; none had any clinical evidence of periodontitis (probing depth ≤ 3 mm, and the percentage of sites with a bleeding index of ≥ 3 was less than 10%). None of the studied subjects had any systemic disease or were taking any medication known to affect the periodontal status. All subjects included had not taken any antibiotics during the past 3 mo and had not accepted any periodontal therapy within the previous 1 year. Pregnant women were excluded. Each subject completed a questionnaire. The questions on the questionnaire included age, weight, height and smoking status (current = smokers, never and former = nonsmokers). The body mass index was calculated. The present study was conducted after obtaining informed consent from all subjects, and was approved by the Ethics Committee of Peking University Health Science Center.

Clinical parameters

Full-mouth periodontal examinations were conducted using a Williams periodontal probe. Probing depth was measured from the gingival margin to the base of the crevice/pocket at six sites (mesial, distal and middle sites of both buccal and lingual sides) of all teeth except third molars. The periodontal attachment level was measured from the cemento–enamel junction to the base of the crevice/pocket at the respective site. The site with both probing depth > 6 mm and attachment level > 5 mm was defined as a severe site. The percentage of severe sites was calculated. These examinations were carried out by two skillful practitioners. For each patient, a set of full-mouth periapical radiographs was taken.

Blood collection and assessment

Blood samples were collected by standard venipuncture using EDTA-containing tubes, between 8:00 and 10:00 h. Plasma was separated and immediately stored frozen at −70°C until required for analysis. Plasma levels of C-reactive protein were measured using a high-sensitivity C-reactive protein enzyme-linked immunosorbent assay and a commercially available kit (Diagnostic System Laboratories, Inc., Webster, TX, USA); the lower limit of detection of C-reactive protein was 0.18 mg/L. Plasma levels of interleukin-6 were determined using a commercially available enzyme-linked immunosorbent assay kit (R&D Systems, Inc., Minneapolis, MN, USA); the lower limit of detection of interleukin-6 was 0.16 pg/mL. These assays were performed according to the manufacturer's protocol.

Statistical methods

C-reactive protein and interleukin-6 values were $\log_{10}$ transformed as a result of their non-normal distributions, and were compared using analysis of covariance, controlling for age, gender, body mass index and smoking. Group comparisons were performed using the Mann–Whitney test in cases of extreme, non-normal distributed variables. The chi-square test was used for frequency data.

Spearman correlation analyses were conducted between plasma levels of C-reactive protein, interleukin-6 and clinical parameters, peripheral blood cells and protein variables respectively. Multiple linear regression analysis was performed with log-transformed C-reactive protein and interleukin-6 levels as the outcome. Potential confounders included in these analyses were age, gender, body mass index and smoking.

Results

The characteristics and clinical parameters of the aggressive periodontitis

Table 1. Study population characteristics in aggressive periodontitis (AgP) and control groups (mean ± SD)

	AgP (n = 84)	Control (n = 65)
Age	27.8 ± 5.76	26.8 ± 4.69
Gender (female/male)	46/38	44/21
BMI (kg/m^2)	21.9 ± 3.52	21.1 ± 1.93
Current smoker (%)[a]	13	0
Probing depth (mm)[b]	4.87 ± 1.06	1.94 ± 0.28
Attachment level (mm)[b]	4.96 ± 1.79	0.05 ± 0.14
Severe sites %[a]	34.6 ± 21.1	0

BMI, body mass index (kg/m^2).
[a]Significant differences between groups using the chi-square analysis, $p < 0.01$.
[b]Significant differences between groups using the Mann–Whitney test, $p < 0.01$.

and control groups are summarized in Table 1, which shows that the two groups were significantly different with respect to probing depth, attachment level and severe sites percentage. As one would expect, generally more severe clinical indices were observed in the aggressive periodontitis group and less severe indices in the control group. Eleven patients with aggressive periodontitis were current smokers, but none of the control subjects smoked. No significant differences were observed between the two groups regarding body mass index, gender and age.

The median value of plasma C-reactive protein in patients with aggressive periodontitis was 1.87 mg/L, whereas the median value of plasma C-reactive protein in the control group was 0.52 mg/L (Fig. 1A). The levels of plasma interleukin-6 in patients with aggressive periodontitis and control subjects, as seen in Fig. 1B, were 1.20 pg/mL and 0.08 pg/mL respectively. The differences of C-reactive protein and interleukin-6 between the two groups were statistically significant after adjustment for body mass index, gender, age and smoking (p = 0.000, p = 0.000, respectively).

Correlation analysis showed that log C-reactive protein was significantly associated with the clinical variables, plasma albumin, triglyceride values and leukocyte count (Table 2). Table 2 also demonstrated that log interleukin-6 was significantly correlated with the clinical variables, fasting blood glucose and leukocyte count.

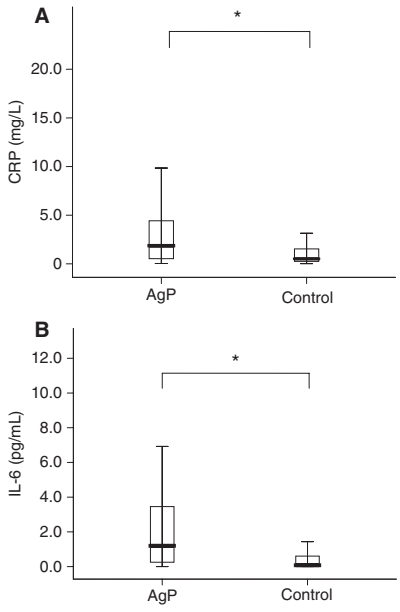

Fig. 1. Box-plots for C-reactive protein (A) and interleukin-6 (B) in aggressive periodontitis and control groups. The horizontal line inside the box represents the median (50th percentile). Statistical analyses were performed on the log-transformed values. *Compared with the control group, based on analysis of covariance, controlling for age, gender, body mass index and smoking. AgP, aggressive periodontitis.

To explore in greater detail the relationships between these two inflammatory mediators and the evaluated values, a multiple linear regression analysis was used. Significant variables in the Spearman correlation analysis were entered in the models. In addition, age, gender, body mass index and smoking were entered in order to adjust for these possible confounding variables. The results of these adjusted models indicated that severe sites percentage and albumin values were significantly related to log C-reactive protein following correction for other variables (Table 3). Table 4 showed that smoking, periodontal diagnosis, fasting blood glucose and leukocyte count were significantly correlated with log interleukin-6.

Discussion

The present study showed significant elevation of C-reactive protein and interleukin-6 in patients with aggressive periodontitis compared with control subjects. The aggressive periodontitis subjects of this study were ascertained using age of disease onset as a diagnostic criterion, and thus the group displays a younger age range than seen in studies of chronic periodontitis. It is reported that the plasma levels of C-reactive protein and interleukin-6 tend to increase with age (13,14), and the incidence of cardiovascular diseases and type 2 diabetes was also associated with age (15,16). Aggressive periodontitis is a relatively early onset type of periodontitis, which has a lower age-related effect on the

Table 2. Associations between log interleukin-6 (Log IL-6) and evaluated variables (n = 149)

	Log CRP		Log IL-6	
	R	p	r	p
PD	**0.410**	**0.000**	**0.396**	**0.000**
AL	**0.390**	**0.000**	**0.421**	**0.000**
Severe sites %	**0.422**	**0.000**	**0.421**	**0.000**
Albumin	−0.236	**0.004**	−0.105	0.240
Cholesterol	−0.017	0.841	−0.016	0.854
Triglycerides	**0.266**	**0.001**	0.106	0.232
Fasting glucose	0.113	0.171	**0.183**	**0.038**
Leukocyte	**0.192**	**0.030**	**0.308**	**0.001**

AL, attachment level; PD, probing depth.
Bold numbers indicate statistically significant difference ($p < 0.05$).

Table 3. Multiple regression of associations between log C-reactive protein and evaluated variables ($n = 149$)

	Standard β	*t*	*p*-value
Gender	−0.360	−4.459	0.000
Severe sites %	0.322	3.647	0.000
Albumin	−0.239	−2.682	0.008
Whole model	$R^2 = 0.351$	$F = 19.964$	0.000

Table 4. Multiple regression of associations between log interleukin-6 and evaluated variables ($n = 149$)

	Standard β	*t*	*p*-value
Smoking	0.182	2.231	0.028
Diagnosis	0.356	4.229	0.000
Fasting glucose	0.202	2.529	0.013
Leukocyte	0.252	3.103	0.002
Whole model	$R^2 = 0.344$	$F = 13.892$	0.000

plasma levels of C-reactive protein and interleukin-6 and on systemic health than chronic periodontitis. Therefore the elevation of C-reactive protein and interleukin-6 in relatively young subjects of the present study may correlate with periodontal destruction rather than with age increase.

Our results confirmed the hypothesis that aggressive periodontitis is related to significantly elevated C-reactive protein levels. The patients with aggressive periodontitis had significantly increased levels of C-reactive protein (1.87 mg/L) compared with control subjects (0.52 mg/L), suggesting that the chronic periodontal inflammatory process in aggressive periodontitis may result in increased levels of C-reactive protein. Moreover, an interesting finding in our study was that plasma levels of albumin are negatively associated with elevated C-reactive protein concentrations after correcting for confounders. As albumin is another important acute-phase protein that shows decreasing levels with inflammation, the association of albumin and elevated C-reactive protein levels further confirmed that aggressive periodontitis may be related to the acute-phase response of liver and subsequent changes in the levels of C-reactive protein and albumin.

Elevated levels of C-reactive protein have been reported in relation to several risk factors for cardiovascular diseases (17). Thus, in the statistical analysis we included age, gender, smoking, body mass index and plasma levels of cholesterol and triglycerides. Based on the results of the multiple linear regression analysis shown in Table 3, it can be deduced that the current finding of elevated inflammatory markers in aggressive periodontitis are significant in addition to other important confounding factors associated with elevated levels of C-reactive protein.

The present result, of elevated C-reactive protein levels in generalized aggressive periodontitis, confirms the results of the study by Salzberg *et al.* (10). In that study, patients with generalized aggressive periodontitis had significantly increased levels of C-reactive protein (3.72 mg/L) compared to the subjects with localized aggressive periodontitis (2.57 mg/L) and no periodontitis (1.54 mg/L). The C-reactive protein levels in our study were 1.87 and 0.52 mg/L in patients with generalized aggressive periodontitis and control subjects, respectively, which are lower than those reported in the study of Salzberg *et al.* One possible explanation for this may be the differences in smoking rates and race between the two studies. The frequency of current smokers in the study of Salzberg *et al.* was 57%, much higher than the frequency of 13% reported in the present study. Smoking is known to be an important determinant of the host's ability to initiate an effective humoral response to infection (18,19). Race/ethnicity has also been found to affect the levels of C-reactive protein (20). About 61% of subjects with generalized aggressive periodontitis in Salzberg's study were Black, while all subjects in our study were of the Chinese Han race. The levels of C-reactive protein were reported to be lower in Asian populations (21,22) than in populations of western countries. Pitiphat *et al.* showed that in a Thai population, subjects with generalized periodontitis had higher serum C-reactive protein levels than controls (1.78 vs. 0.25 mg/L) (23). Our data are similar to the result obtained for Thai people, indicating a lower range of systemic C-reactive protein levels in Asian populations.

The levels of interleukin-6 were found to correlate with periodontal diagnosis and leukocyte count after correcting for confounding factors. Elevated numbers of leukocytes in periodontitis have previously been observed (5) and interleukin-6 in blood is mainly released by circulating leukocytes. These results are consistent with the interpretation that higher levels of interleukin-6 may be produced by enhanced numbers of leukocytes in patients with aggressive periodontitis.

C-reactive protein is one of the major acute-phase proteins, which are synthesized primarily by the liver in response to pro-inflammatory cytokines. The concentration of C-reactive protein increases with inflammation (24). Recently, C-reactive proteins have been found to activate complement in damaged vessel walls and to promote the formation of foam cells during the initiation of atheroma formation (25,26). Interleukin-6 is an important activator of C-reactive protein production (27) and is a key pro-inflammatory and immune-modulatory cytokine, secreted mainly by monocytes, macrophages and T lymphocytes recruited to sites of infection or inflammation. Interleukin-6 has pro-inflammatory properties and procoagulant effects, and these properties are likely to play roles in the pathogenesis of coronary syndromes (28). The elevation of C-reactive protein and interleukin-6 in the present study suggests a plausible biologic mechanism underlying the association

between periodontitis and cardiovascular diseases.

Another important finding is that fasting blood glucose was independently associated with elevated plasma levels of interleukin-6. A recent study highlighted the fact that interleukin-6 may affect glucose metabolism (29). Several population-based studies have shown that increased serum levels of interleukin-6 are positively correlated with insulin resistance (4,30,31). An acute *in vivo* increase of systemic interleukin-6 levels in healthy human subjects by injection of recombinant human interleukin-6 has been reported to increase plasma glucose levels (32). The majority of animal experiments also indicate that chronic elevation of interleukin-6 can mediate insulin resistance (33,34). Those elevations of interleukin-6 may act through insulin receptor substrate 1 and protein kinase B (also known as Akt) signaling pathways to promote hepatic insulin resistance, and thus exacerbate the syndrome (35,36). The observation of a positive association between plasma interleukin-6 levels and fasting blood glucose in this study provides further evidence to support this notion. Furthermore, our previous study of the same cohort of patients observed a higher fasting blood glucose level in patients with aggressive periodontitis than in the controls. All these observations, including the increased levels of fasting blood glucose (11), elevated concentrations of interleukin-6, and correlation between the fasting blood glucose level and concentration of interleukin-6, may partially explain the influence of periodontitis on diabetes.

The present study demonstrated that the levels of C-reactive protein and interleukin-6 were elevated in the plasma of patients with aggressive periodontitis in China, after controlling for the important confounders. Furthermore, it provides the first evidence that the concentration of interleukin-6 is positively related to fasting blood glucose level in patients with aggressive periodontitis. These findings may represent a contribution of periodontal infections to cardiovascular diseases and diabetes in relatively young individuals.

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