**Association between Plasma Leptin Level and Systemic Inflammatory Markers in Patients with Aggressive Periodontitis**

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**Abstract**

**Background:** Increasing evidence supports an association between periodontitis and systemic diseases. Leptin is involved both in the energy metabolism and inflammatory processes and is suggested to be a link between periodontal infection and systemic health. The present study aimed to evaluate the peripheral leptin concentration in patients with aggressive periodontitis (AgP) and to explore the relationship between leptin and systemic inflammation.

**Methods:** Ninety patients with AgP visiting the Clinic of the Periodontology Department, Peking University School and Hospital of Stomatology between July 2001 and May 2006, and 44 healthy controls (staff and student volunteers in the same institute) were recruited. Plasma levels of leptin and inflammatory cytokines including interleukin (IL)-1β, IL-6, tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) were measured by enzyme-linked immunosorbent assay. Correlation and multiple linear regression analysis were performed to analyze the association between plasma leptin level and other variables.

**Results:** Plasma leptin level of AgP group was significantly higher than that of the control group (19.7 ± 4.4 ng/ml vs. 7.5 ± 1.3 ng/ml, \( P < 0.01 \)). After controlling for age, gender, and body mass index, positive correlation was observed between plasma leptin concentration and log-transformed levels of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α and CRP), and the partial correlation coefficients ranged from 0.199 to 0.376 (\( P < 0.05 \)). Log-transformed IL-1β and IL-6 levels entered the final regression model (standardized \( \beta \) were 0.422 and 0.461 respectively, \( P < 0.01 \)).

**Conclusions:** Elevated plasma leptin concentration may be associated with increased systemic levels of inflammatory markers in AgP patients.

**Key words:** Aggressive Periodontitis; Inflammatory Mediators; Leptin

**INTRODUCTION**

During the last decades, evidence has emerged to support the relationship between periodontal infection and systemic conditions including cardiovascular diseases, diabetes, obesity and metabolic syndrome. Unlike the most common form of chronic periodontitis (CP), aggressive periodontitis (AgP) develops early in life, progresses faster, and presents more destructive clinical features. It has been indicated that systemic factors play a greater role in the etiology of AgP, which in turn exert greater effects on systemic conditions. As expected, higher serum levels of inflammatory mediators, such as interleukin (IL)-17, tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP), have been detected in patients with CP compared to controls, which became even higher in AgP patients, even if whose clinical parameters were less severe. The increased systemic inflammation burden has been suggested to be a possible link between periodontal disease and systemic health. For example, pro-inflammatory cytokines were shown to play important roles in the development of dyslipidemia and impaired glucose tolerance, which have been considered as risk factors for many systemic diseases.

Leptin, since discovered in 1994, has provided important insights into the intricate network linking nutrition, metabolism, and immune homeostasis. This 16-kDa adipose tissue-derived molecule plays important roles in regulating lipid and glucose metabolism, and elevated plasma leptin concentrations have been suggested as an independent risk factor for cardiovascular diseases. In recent years, clinical studies indicated an association
between leptin and periodontal infection. Serum leptin concentration was reported to be significantly elevated in CP patients compared with periodontal healthy subjects, and was positively correlated with periodontal parameters including probing depth and clinical attachment loss, and could be effectively reduced by periodontal treatment. These results suggested that the systemic leptin level could be greatly influenced by the periodontal conditions, and is a potential mediator between periodontitis and systemic health.

We and others have shown elevated systemic inflammatory markers including peripheral leukocytes counts, CRP and IL-6 in patients with AgP. Our group further independently found that plasma leptin concentration significantly increased in AgP patients. However, evidence is still lacking about whether there is an association between the circulating leptin concentration and the systemic inflammation in patients with AgP. The aim of the present study was to evaluate the change of leptin concentration in peripheral blood in patients with AgP, and to explore the relationship between leptin and systemic inflammation markers.

**METHODS**

**Study population**

The present study was conducted with the written informed consent of all subjects and was approved by the Ethics Committee of the Peking University Health Science Center. Ninety patients with generalized AgP (33 males and 57 females), aged 14–42 years, were recruited from the Clinic of the Periodontology Department, Peking University School and Hospital of Stomatology between July 2001 and May 2006. The diagnosis criteria were defined according to the classification developed at the International Workshop for a Classification of Periodontal Diseases and Conditions in 1999: the onset of periodontal disease generally occurred at <35 years of age, and there were at least eight teeth with probing depth (PD) >6 mm and with radiographic evidence of alveolar bone loss. At least three of these teeth were not first molars or incisors. Other factors were also considered: familial aggregation, rapid progression, and the relationship between local factors and periodontal destruction.

Forty-four healthy volunteers (16 males and 28 females), aged 20–48 years, were recruited from the staff and students of the Peking University School and Hospital of Stomatology. The inclusion criteria were no site with clinical attachment loss, no site with PD > 3 mm, no bone loss on radiographs, and less than 10% of sites with bleeding on probing.

The exclusion criterions included systemic diseases, smoking, periodontal therapy within the previous year, antibiotics intake within the previous 3 months, and pregnancy. By their own acknowledgment, all study subjects were free of systemic diseases and were not taking any medication known to affect periodontal status. All recruited subjects accepted a comprehensive blood examination and were referred to physicians when necessary.

All subjects belonged to the Han race, which makes up the majority of the Chinese population. Each study subject filled out a questionnaire that noted general background (including weight and height), medical and dental care history, oral hygiene habits, and social status. The body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

**Blood collection and assessment**

According to our previous results, systemic inflammatory markers including white blood cell (WBC) count, IL-1β, IL-6, CRP and TNF-α may be correlated with periodontal infection. Serum protein variables such as albumin (ALB)/globulin ratio (A/G) were also associated with the severity of periodontal destruction. These variables were thus included in the present study.

A peripheral blood sample was obtained from each fasting examinee by venipuncture between 8:00 a.m. and 10:00 a.m., and was divided into two tubes. One tube contained ethylenediaminetetraacetic acid (EDTA) and was used for blood cell analysis by hematology analyzers (SYSMEX KX-21, Sysmex, Kobe, Japan); the other did not contain EDTA and was used for serum protein analyses by a biochemical analyzer (HITACHI 7060, Hitachi, Tokyo, Japan). Blood cell analysis included WBC count, neutrophil count, and lymphocyte count. Serum protein parameters included total protein, ALB, globulin, and A/G.

Plasma samples were obtained from EDTA-containing tubes and were separated and immediately stored frozen at −70°C until required for analysis. Plasma levels of leptin, IL-1β and IL-6 were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R and D Systems, Inc., Minneapolis, MN, USA); and the lower limits of detection were 7.8 pg/ml, 1 pg/ml and 0.16 pg/ml respectively. Plasma level of CRP was measured using a commercially available ELISA kit (Diagnostic System Laboratories, Inc., Webster, TX, USA); the sensitivity of this kit was 0.18 mg/L. Plasma levels of TNF-α were measured using a commercially available ELISA kit (Bender Medsystems, Inc., Vienna, Austria) and the lower limit of detection was 0.13 pg/ml. These assays were performed according to the manufacturer’s protocol.

**Statistical analysis**

Plasma leptin levels were analyzed with the analysis of covariance model, controlling for age, gender, and BMI as confounders. Partial correlation coefficients between leptin and other parameters including clinical values, hematoletic parameters, and pro-inflammatory cytokines were determined, controlling for the potential confounders mentioned above. Multiple linear regression analysis was performed with plasma leptin value as the outcome. The values of age, BMI, plasma leptin level, blood cell and serum protein variables were described by mean ± standard deviation (SD). Plasma levels of CRP, IL-6, IL-1β and
TNF-α were described by median and interquartile range, and were log_{10} transformed for the correlation and regression analyses as a result of their nonnormal distributions. SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was used for analyses, and $P < 0.05$ was considered as statistically significant.

**Results**

The population variables and plasma leptin levels were shown in Table 1. Mean ages of control and AgP groups were 25.6 ± 3.8 years and 26.2 ± 4.9 years, respectively. There was no difference in the mean values for age and BMI between the two groups and no significant difference for gender distribution. Mean plasma leptin level of AgP group was 19.7 ± 4.4 ng/ml, significantly higher than that of the control group (7.5 ± 1.3 ng/ml, $P < 0.01$). Table 1 also presented the results of blood cell analysis and serum protein assessment. Compared with the control group, significantly higher WBC and neutrophil counts were observed in the AgP group; the ALB and A/G were significantly lower in the AgP group than in the control group ($P < 0.01$).

The results of ELISA measurement were presented in Table 2. Plasma levels of CRP, IL-1β, IL-6 and TNF-α were significantly higher in AgP patients compared with controls ($P < 0.01$).

Results of partial correlation analyses were shown in Tables 3-5. When all the subjects in both groups were pooled for analyses, after controlling for age, gender and BMI, plasma leptin level was significantly positively correlated with WBC and neutrophil counts as well as log-transformed levels of pro-inflammatory cytokines, and the partial correlation coefficients ranged from 0.199 to 0.376 ($P < 0.05$). A negative correlation was observed between plasma leptin level and A/G as well as ALB, and the $r$ values were −0.246 and −0.198 respectively ($P < 0.01$). Multiple linear regression analysis was performed to explore the relationship between leptin and other parameters. Gender, age, BMI and significant variables in the partial correlation analysis were evaluated. Using stepwise method, log-transformed IL-1β and IL-6 entered the final model, and the standardized $β$ values were 0.422 and 0.461, respectively ($P < 0.001$). The adjusted $r^2$ value of this model was 0.376.

**Discussion**

Although numerous research have investigated the serum leptin levels in patients with gingivitis and CP, few evidence...
is present about that in patients with AgP, which is a distinct form of periodontitis and characterized by early onset and rapid and severe periodontal destruction, as well as its relationship with systemic inflammatory markers. As far as we knew, this study provided the first evidence about the positive association between plasma leptin levels and systemic inflammatory markers including WBC and neutrophil counts, IL-1β and IL-6 in patients with AgP.

The mechanism through which periodontitis is associated with circulating leptin level is still unclear. However, previous evidence showed that resident cells in the inflammatory periodontal tissue could be an important source of inflammatory mediators, among which IL-1β and IL-6 were both involved. Leptin, with a striking structural similarity with IL-6, shares regulatory and functional similarity with IL-6 as well. Another research from our group has shown that healthy resident cells in periodontal tissues, such as gingival epithelial cells, gingival connective cells and periodontal ligament cells, all expressed leptin to some extent both in vivo and in vitro. Thus, the mechanism contributing to the up-regulation of plasma IL-6 levels in periodontitis may propose the same effect on the change of plasma leptin levels. On the other hand, the released lipopolysaccharide, TNF and IL-1 from periodontal infection may increase the adipose tissue leptin mRNA expression, hereby circulating leptin levels more significantly as suggested by animal studies. In consistent, plasma leptin level positively correlated with the values of systemic inflammation markers including WBC and IL-6 levels, which have been observed in AgP patients according to our present and previous studies.

Previous studies have observed that serum leptin levels increased as the severity of periodontal inflammation progressed, which were lowest in the controls, moderate in patients with chronic gingivitis and highest in those with CP. Our previous study found that the plasma leptin level in AgP reached 20 ng/ml on average, even higher than the reported 12 ng/ml in CP with comparable PD. The rise of serum leptin level above 10 ng/ml is considered as a risk factor for cardiovascular disease. Preliminary evidence has shown the elevated serum leptin level in CP was associated with type 2 diabetes and acute myocardial infarction. Thus, it is reasonable to suspect that the even higher serum leptin levels in patients with AgP may provide a stronger interrelationship between local infection and systemic conditions.

In summary, plasma leptin levels may be associated with the inflammatory cytokines from periodontal infection, especially in an aggressive form. These results suggested that leptin could be a potential connection between local infection and systemic health. However, the correlation coefficients were relatively low (<0.4). One of the explanations is that other factors that may affect the circulating leptin level such as body fat percentage and lifestyles were not considered in our study. Within the limitation of the present study, elevated plasma leptin concentration was associated with increased systemic levels of inflammatory markers, especially IL-1β and IL-6, in AgP patients after controlling for age, gender, and BMI.

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