Lipoprotein-associated Phospholipase A2 and Serum Lipid Levels in Subjects with Chronic Periodontitis and Hyperlipidaemia

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**Objective:** To evaluate the relationships between clinical periodontal parameters and levels of lipoprotein-associated phospholipase A2 (Lp-PLA2) and lipid profile markers in subjects with or without hyperlipidaemia.

**Methods:** Forty chronic periodontitis (CP) subjects with hyperlipidaemia (CP/HPL group), 40 systemically healthy CP subjects (CP group) and 20 systemically and periodontally healthy subjects (control group) were enrolled. The clinical periodontal parameters, the serum concentrations of Lp-PLA2, lipid profiles including total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and white blood cell (WBC) counts were determined and compared between different groups. Linear regression analysis was performed to identify the contributing factors of Lp-PLA2.

**Results:** Serum Lp-PLA2 level in the CP/HPL group and the CP group was significantly higher than in the healthy group. TC and TG levels in the CP/HPL group were higher than in the CP and control groups. No difference was observed for levels HDL-c and LDL-c and WBC counts among the groups. Linear regression analysis showed that the serum level of Lp-PLA2 was positively associated with bleeding on probing and WBC counts.

**Conclusion:** Elevated level of Lp-PLA2 is associated with periodontal inflammation, indicating that periodontal treatment could reduce the risk of cardiovascular disease in CP subjects with hyperlipidaemia.

**Key words:** periodontitis, hyperlipidaemia, lipoprotein-associated phospholipase A2

Chronic periodontitis (CP), a chronic infection, has been reported to be related to the pathogenesis of atherosclerosis. Meta-analysis of the relationship between periodontal disease and cardiovascular events has showed that subjects with periodontal diseases have higher odds of developing cardiovascular disease1,2. Hyperlipidaemia is one of the traditional risk factors for cardiovascular disease (CVD), leading to focal accumulation of lipids beneath the arterial intima3-5. The factor linking periodontitis, lipid metabolism and CVD might be inflammation. Studies focused on the relationship between periodontitis and serum lipids profiles produced controversial results6-11. Certain lipid levels were associated with gingival inflammation or deep pocket depth, and subjects with hyperlipidaemia had more severe periodontal status compared with systemically healthy controls12-14. In addition, non-surgical periodontal treatment could improve serum lipid levels in subjects with hyperlipidaemia15-17. The human lipoprotein-associated phospholipase A2 (Lp-PLA2) is a serine-dependent, Ca2+-independent enzyme. It hydrolyses oxidised phospholipids, yielding lysophosphatidyl choline and oxidised free fatty acids, which could lead to inflammatory cell chemotaxis, endothelial cell dysfunction and smooth muscle cell...
apoptosis in CVD\textsuperscript{18-20}. Epidemiological studies have indicated that elevated Lp-PLA2 levels are associated with increased risk of CVD, so it has been regarded as an independent risk indicator for CVD emergencies\textsuperscript{21-25}. Periodontal disease is an inflammatory disease that can induce local and systemic elevations of pro-inflammatory cytokines; such as tumour necrosis factor-\alpha and interleukins 1\beta and 6\textsuperscript{26-29}. As Lp-PLA2 is closely related to infection and inflammation, so Lp-PLA2 may influence the development and progress of periodontitis. However, little information is available regarding the relationship between Lp-PLA2 and periodontitis\textsuperscript{30-32}.

The aim of the present study was to evaluate the serum Lp-PLA2 level and lipid profiles in CP subjects with or without hyperlipidaemia, in order to find the relationship between clinical periodontal parameters and Lp-PLA2 level and lipid profiles.

Materials and methods

Study subjects

This study was approved by the Institutional Review Board of Health Science Center, Peking University, P.R. China. The subjects were enrolled consecutively from patients attending the periodontal clinic of the Peking University School of Stomatology. In total, 40 CP subjects with hyperlipidaemia (CP/HPL group), 40 systemically healthy CP subjects (CP group) and 20 systemically and periodontal healthy subjects (control group) were enrolled in the study. The subjects were all above 40 years old. Written informed consents were obtained from the subjects.

The inclusion criteria for the study were as follows. (1) For periodontal disease subjects, each patient had at least 24 teeth, more than 30% of teeth had probing depth (PD) \(\geq 4\) mm and clinical attachment loss (CAL) \(\geq 4\) mm, as well as alveolar bone loss of over 30% of the root length, determined using full-mouth periapical radiographs. (2) For hyperlipidaemia subjects, in order to identify subjects with pathological lipid values, the following cut-off points were used: total cholesterol (TC) \(> 5.2\) mmol/l; triglyceride (TG) \(> 1.7\) mmol/l; and low-density lipoprotein cholesterol (LDL-c) \(> 3.1\) mmol/l\textsuperscript{33}. Subjects who meet any of these three criteria will be diagnosed as hyperlipidaemic.

The exclusion criteria were as follows: (1) subjects had any other systemic diseases affecting lipid metabolism (such as liver diseases, diabetes mellitus, metabolic syndrome or other endocrine diseases, and CVD); (2) subjects had taken any medicine for anti-hyperlipidaemia within 3 months prior to the study; (3) subjects had taken any antibiotics within 4 weeks prior to the study; (4) subjects had periodontal treatment history within prior 6 months; (5) subjects currently receiving hormone replacement treatment; and (6) subjects less than 40 years of age.

Clinical periodontal examination

All subjects underwent a full-mouth periodontal examination prior to periodontal therapy (baseline). The examination involved probing of six sites (distofacial, facial, mesiofacial, distolingual, lingual and mesiolingual) per tooth (third molars excluded), carried out by a single, calibrated periodontist using a Williams manual periodontal probe. The periodontal parameters assessed were as follows. (1) Probing depth (PD), the distance of the free gingival margin to the base of the probable pocket, recorded to the nearest mm. (2) Clinical attachment loss (CAL), as measured between the bottom of the pocket and the cementoenamel junction recorded to the nearest millimetre. (3) Bleeding on probing (BOP): six sites for each tooth were examined, with a positive score if bleeding occurred immediately after probing. (4) Plaque index (PLI) (Silness and Löe 1964): 0 = no plaque; 1 = appears clean but plaque removed from gingival margin with a probe; 2 = visible plaque along gingival margin; 3 = abundant plaque along gingival margin.

Smoking history and body mass index (BMI)

Smoking history was collected for all subjects. The body mass and height of all subjects were measured by the standard scale; BMI was calculated by the formula:

\[
\text{BMI} = \frac{\text{body mass (kg)}}{\text{body height (m)}}^2
\]

Laboratory testing

Blood was obtained before treatment in the morning, after fasting for at least 12 h. Serum was isolated from each blood sample and frozen at -80 °C for later analysis. White blood cells (WBCs) were counted and TC, TG and high-density lipoprotein cholesterol (HDL-c) were measured by enzymatic assay using the 7600-020 Automatic Analyzer (Hitachi, Japan). LDL-c was calculated using Friedewald’s formula:

\[
\text{LDL-c} = \text{TC} - [\text{HDL-c} + (\text{TG}/5)].
\]
Serum level of Lp-PLA2 was determined using a commercially available ELISA kit (PLACTM Test) (diaDexus, South San Francisco, CA, USA). Each sample was analysed in duplicate.

Statistical analysis

The data were presented as mean ± standard deviation. Analysis of variance (ANOVA) was used to examine the difference among the groups, followed by post hoc Bonferroni correction. In order to identify whether general characteristics, periodontal parameters or biochemical indices were associated with Lp-PLA2, multiple linear regression analysis was performed. Lp-PLA2 level was used as the dependent variable, and the explanatory variables were as follows: age, gender, BMI, history of smoking, WBC counts, PD, CAL, PLI, BOP and lipid markers. A $P$-value $<$ 0.05 was considered to be statistically significant.

Results

General characteristics in different groups

As shown in Table 1, the CP/HPL group had a higher age than the CP group ($P < 0.001$) and higher BMI index than the control group ($P < 0.001$); the CP group had a similar age to the control group but a higher BMI ($P < 0.001$). The gender distribution and smoker percentages were not significantly different between the groups.

Periodontal parameters in different groups

As shown in Table 2, the CP/HPL group had worse CAL than the CP group ($P = 0.005$); all clinical parameters in the control group were significantly better than in the CP/HPL and CP groups ($P < 0.017$).

Lp-PLA2 and lipid levels in different groups

As shown in Table 3, for serum Lp-PLA2 and WBC counts, the values for the CP/HPL and CP groups were significantly higher than for the control group (for Lp-PLA2, $P < 0.001$; for WBC counts, $P = 0.001$); there was no significant difference in Lp-PLA2 and WBC counts between the CP/HPL group and the CP group. TC and TG levels in the CP/HPL group were higher than that in the CP and control groups ($P < 0.001$). The HDL-c level in the CP/HPL group was lower than that in the CP and control groups ($P < 0.001$). LDLC levels showed no difference between the groups.
Multiple linear regression analysis

Multiple linear regression analysis showed that the serum level of Lp-PLA2 was positively associated with BOP and WBC count ($P < 0.001$, $\beta = 0.370$; $P = 0.032$, $\beta = 0.211$, respectively) and negatively associated with age ($P = 0.017$, $\beta = -0.220$).

Discussion

Lp-PLA2 is regarded as an important biomarker for predicting atherosclerosis risk$^{21-25}$. Only three previous studies have reported on the relationship between Lp-PLA2 level and periodontitis$^{30-32}$. In the present study, we evaluated the association between clinical periodontal parameters, levels of Lp-PLA2 and lipid profiles in CP subjects with or without hyperlipidaemia. Levels of Lp-PLA2 and WBC counts were significantly higher in the CP and CP/HL groups compared with the healthy control group. Linear regression analysis showed that level of Lp-PLA2 was positively associated with BOP and WBC counts. It was demonstrated that periodontal infection could lead to systemic inflammation which consequently increased the Lp-PLA2 level. Periodontal treatment might be a possible way to reduce the risk of CVD in some CP subjects with hyperlipidaemia.

Lp-PLA2 generates pro-inflammatory molecules from oxidised LDL-c. Elevated Lp-PLA2 concentrations are associated with the presence of unstable CVD, independent of various biochemical markers$^{25}$. Lösch et al reported that moderate periodontitis is associated with elevated activity of Lp-PLA2 (10% higher than in healthy controls)$^{30}$, and that periodontal parameters such as BOP, PD and CAL are positively correlated with the serum activity of Lp-PLA2$^{31}$. Fentoğlu et al conducted research in hyperlipidaemia subjects with different periodontal statuses; they found the gingivitis group with hyperlipidaemia to have higher serum Lp-PLA2 and high-sensitivity C-reactive protein (hsCRP) levels compared with those of the systemically healthy gingivitis subjects. Serum Lp-PLA2 and hsCRP levels were significantly correlated with TC:HDL-c ratio, the gingival index, PD and BOP (%) in the hyperlipidaemia group$^{32}$. In our study, the Lp-PLA2 levels in CP and CP/HL subjects were about 25% to 30% higher than in healthy controls and Lp-PLA2 level is closely associated with BOP and WBC count. All these studies indicated that CP could increase the Lp-PLA2 level.

Lösch et al compared plasma lipid levels in 39 subjects with moderate periodontal disease with 40 age- and sex-matched controls and observed that TC, LDL-c and TG are significantly higher in periodontitis patients, by about 8%, 13% and 39%, respectively$^7$. Shi et al reported higher serum levels of TC in CP subjects (4.61 ± 1.23 mmol/l) compared with healthy controls (4.49 ± 0.78 mmol/l)$^{11}$. In our study, TC and TG levels in the CP/HL group were higher than in the CP and healthy control groups ($P < 0.001$), and HDL-c levels in the CP/HL group were lower than in the CP and healthy control groups. Fentoğlu et al conducted research in 51 subjects with hyperlipidaemia and 47 normolipidaemic subjects. They found the mean values of PLI, PD, CAL and BOP for the hyperlipidaemic group were significantly higher than those for the control group; plasma TC, TG and LDL-c were significantly and positively associated with PLI, PD, BOP and CAL; and HDL-c was significantly, but negatively, associated with CAL$^{13}$. In our study, the CAL in CP/HL group was significantly worse than in the CP group. One possible mechanism for this lies in the bidirectional association between periodontitis and hyperlipidaemia. The

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>CP/HPL group (N = 40)</th>
<th>CP group (N = 40)</th>
<th>Control group (N = 20)</th>
<th>Comparison 1 (P)</th>
<th>Comparison 2 (P)</th>
<th>Comparison 3 (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp-PLA2 (μg/l)</td>
<td>30.06 ± 10.96</td>
<td>33.13 ± 12.57</td>
<td>22.19 ± 4.96</td>
<td>0.036</td>
<td>0.003*</td>
<td>&lt; 0.001*</td>
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<tr>
<td>TC (mmol/l)</td>
<td>5.76 ± 0.73</td>
<td>4.61 ± 0.73</td>
<td>4.80 ± 0.58</td>
<td>&lt; 0.001*</td>
<td>&lt; 0.001*</td>
<td>0.590</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.80 ± 0.60</td>
<td>1.12 ± 0.38</td>
<td>0.98 ± 0.42</td>
<td>&lt; 0.001*</td>
<td>&lt; 0.001*</td>
<td>0.239</td>
</tr>
<tr>
<td>HDL-c (mmol/l)</td>
<td>1.24 ± 0.41</td>
<td>1.46 ± 0.40</td>
<td>1.65 ± 0.53</td>
<td>0.008*</td>
<td>&lt; 0.001*</td>
<td>0.106</td>
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<tr>
<td>LDL-c (mmol/l)</td>
<td>2.85 ± 0.94</td>
<td>2.68 ± 0.70</td>
<td>2.84 ± 0.59</td>
<td>0.378</td>
<td>0.539</td>
<td>0.154</td>
</tr>
<tr>
<td>WBC counts (/l)</td>
<td>6.04 ± 1.02</td>
<td>6.08 ± 0.97</td>
<td>5.22 ± 0.95</td>
<td>0.883</td>
<td>0.002*</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Comparison 1: CP/HPL group versus CP group; Comparison 2: CP/HPL group versus control group; Comparison 3: CP group versus control group.

* Statistically significant.
deterioration of lipid metabolism will be strengthened by bad periodontal indices, and vice versa.

In summary, in this study, levels of Lp-PLA2 were significantly higher in the CP/HPL and CP groups compared with the healthy control group, and the serum level of Lp-PLA2 was positively associated with BOP and WBC count. This might provide a mechanism for reducing the cardiovascular risk of some CP or CP/HPL patients through periodontal treatment.

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References