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Serum Lp-PLA2: as a novel viewpoint in periodontal treatment of hyperlipidaemics

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1. Introduction
Systemic inflammation and lipid deposits play a pivotal role in atherothrombotic inception and progression (1). Lipoprotein-associated acute phase reactants such as C-reactive protein (CRP) (2) and lipoprotein-associated phospholipase A2 (Lp-PLA2) (3) have been shown to increase in acute coronary events.

Taking into account the effects of acute phase reactants, Lp-PLA2 is a major inflammatory marker. Lp-PLA2 has an important role in the pathogenesis of atherosclerosis via enhanced proinflammatory cytokines, oxidation of polyunsaturated fatty acids, and low-density lipoprotein (LDL) cholesterol. It also plays an effective role in endothelial cell dysfunction and cardiovascular and thromboembolic events (3,4).

The effects of Lp-PLA2 on clinical periodontal parameters were first reported by Löshe et al. (5). Individuals with moderate periodontitis had higher Lp-PLA2 levels than periodontally healthy controls. Furthermore, the activity of serum Lp-PLA2 decreased after periodontal therapy in systemically healthy individuals with periodontitis (5).

Another lipoprotein-associated inflammatory mediator is CRP, which may play an important role in LDL oxidation, complement activation, endothelial dysfunction, and atherogenesis (6,7). A relationship between enhanced plasma CRP levels and clinical periodontal parameters has also been reported (8).

Although there are several studies showing the association between periodontal disease and hyperlipidaemia (9–11), there are only 3 studies reporting the effects of periodontal therapy on the metabolic and inflammatory control of lipid metabolism in hyperlipidaemic patients (10,12,13). In our previous
2. Materials and methods

2.1. Study population
This study was conducted by the Internal Medicine and Periodontology Departments of Süleyman Demirel University, Isparta, Turkey. The study was performed in accordance with the principles outlined in the Helsinki Declaration of 1975, as revised in 2000. The study protocol was approved by the Ethics Committee of Süleyman Demirel University (Date: 05.12.2006, Number: 09/11). Informed consent was obtained from all subjects. Data collection was performed from July 2007 to July 2009.

The study population included patients with periodontitis who composed a part of our previous study (15). The participants with periodontitis applied to the Internal Medicine and/or Periodontology Department for systemic, dental, or periodontal examination. The hyperlipidaemic groups were made up of patients receiving a prescribed diet (HD) or a statin antilipaemic drug (HS). The control group was made up of systemically healthy subjects. The study population was subjected to detailed systemic examination to establish the medical status of the subjects.

For the HD group, plasma LDL values were <160 mg/dL and >130 mg/dL. For the HS group, plasma LDL values were >160 mg/dL (16). An antilipaemic therapy that included a lipid-lowering diet and physical activity were recommended by the same physician (BKK) for the HD and HS groups at the beginning of the study. Atorvastatin (10 mg) was also prescribed for the HS group by the same physician (BKK). Demographic characteristics such as age, sex, body mass index (BMI) (kg/m²), and the number of natural teeth were recorded.

Patients with the following criteria were excluded: any systemic diseases such as impaired glucose tolerance, diabetes mellitus, metabolic syndrome or other endocrine disease, renal dysfunction, and cardiovascular disease; antilipaemic treatment (diet or diet and statin therapy) more than 1 month prior the study; any drug treatment (such as hormone replacement, systemic antibiotic, or antiinflammatory agent) within 3 months of the study; any periodontal treatment within 6 months of the study; tobacco use (current smoker or former smoker having quit less than 6 months prior to the study); pregnancy; and alcohol use.

2.2. Periodontal parameters and periodontal treatment
All dental examinations were conducted by a clinician (ÖF). The clinical periodontal parameters included plaque index (PI) (17), gingival index (GI) (18), probing pocket depth (PPD), clinical attachment level (CAL), and percentage of bleeding on probing (BOP%). The measurements were performed mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual with a Williams periodontal probe (Hu-Friedy, Chicago, IL, USA). Wisdom teeth were not evaluated. The clinical diagnosis of chronic periodontitis was based on having at least 4 teeth with a PPD of ≥5 mm and a CAL of ≥2 mm at the same time (19). Subjects with chronic periodontitis who had at least 18 natural teeth were included in the study (20,21).

Systemic and periodontal examinations, which included serum and clinical periodontal parameters, were performed at baseline. Hyperlipidaemic patients underwent diet, physical activity, and/or statin therapy according to a physician’s recommendations (BKK) at the beginning of the study. At the first appointment, intensive oral hygiene instruction including tooth brushing, plaque disclosing, and interdental cleaning as well as full mouth scaling and polishing were performed. Blood samples were collected in the first week after the initial visit for periodontal treatment (1WPT) for the evaluation of serum lipids, Lp-PLA2, and CRP levels. All of the participants then underwent deep scaling and root planing (on a quadrant-by-quadrant basis) by the same clinician (ÖF) with local anaesthesia. Gracey curettes were used to do the periodontal treatment. Systemic and periodontal parameters were reevaluated 2 months after the completion of the nonsurgical periodontal treatment (2MPT). For each patient, the study protocol was completed in 3 months. The design of the study is demonstrated in the Figure. Intraexaminer calibration was provided by an examiner (ÖF) with 85% accuracy.

2.3. Serum samples and laboratory analyses
At baseline (BL), the first week after initial periodontal therapy (1WPT), and 2 months after the completion of periodontal treatment (2MPT), blood samples were collected in vacutainer tubes. The samples were obtained after a 12-h fasting period from an antecubital vein. Serum triglyceride (TRG), total cholesterol (TC), LDL, high-density lipoprotein (HDL) cholesterol, very low-density lipoprotein (VLDL) cholesterol, Lp-PLA2, and CRP levels were evaluated. Biochemical evaluations were
performed in the Laboratory of Clinical Biochemistry, Faculty of Medicine, Süleyman Demirel University. The samples were centrifuged at 4000 rpm for 4 min and the serum was separated. The serum samples were stored at –80 °C until the laboratory analyses were done. For the detection of serum lipid levels, routine enzymatic methods were used. To determine the case and control groups, the cut-off points were: TRG > 200 mg/dL, TC > 200 mg/dL, LDL > 130 mg/dL, HDL < 35 mg/dL, and VLDL > 40 mg/dL. Serum levels of TC, LDL, HDL, VLDL, and TRG were determined by an autoanalyser (Olympus AU 2700, Japan). Serum high-sensitive CRP levels were measured by an electrochemiluminescence immunoassay using a hormone analyser (Immulite 2000, USA). The results are expressed in mg/L. Enzyme-linked immunosorbent assay kits (Cayman Chemical Company, USA) were used to determine the activity of serum Lp-PLA2. The results are expressed mmol min⁻¹ mL⁻¹.

2.4. Statistical analysis
SPSS (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis. The power of this study was above 85% (NCSS/PASS, 2000 Dawson Edition, Kaysville, UT, USA). The Kolmogorov–Smirnov test was used to determine data distribution. For the groups of normally distributed variables, one-way analysis of variance (ANOVA) and t-tests were used. Kruskal–Wallis one-way ANOVA and the Mann–Whitney U test with Bonferroni’s correction were used for nonnormally distributed variables. The differences among the 3 study periods were assessed using the Friedman test (P < 0.05). For post hoc analyses, the Wilcoxon signed ranks test was used (P < 0.0167).

3. Results
The study included 80 individuals aged 30–57 years, with 52 patients with hyperlipidemia aged 30–57 and 28 controls aged 31–54. Table 1 shows the demographic characteristics of the hyperlipidaemic and control groups. The case and control groups did not differ significantly in terms of age or sex (P > 0.05). Five patients in the HS group had been receiving atorvastatin for 1 month prior to the study. For the HS group, atorvastatin (10 mg) was prescribed at the beginning of the study by a physician. The statin dosage of the study population was constant throughout the study. All of the patients complied with the physician’s suggestions (physical activity, diet, and/or antilipaemic drug usage) during the study period.

The periodontal and serum parameters of the study groups are shown in Table 2. In the HS group, BOP% values were significantly higher than in the C and HD groups at baseline (P < 0.001). There were significant differences in the periodontal parameters (PI, GI, BOP%, and PPD) between 2MPT and baseline in the C, HD, and HS groups (Table 3).

There were statistically significant increases in VLDL levels at 1WPT compared to baseline in the C group (P = 0.003). In addition, statistically significant increases were found in TRG levels at 2MPT compared to baseline in the HD and HS groups (P = 0.047 and P = 0.036, respectively). There were insignificant decreases in TC and LDL levels of the HD and HS groups at 1WPT and 2MPT compared to baseline (P > 0.05) (Table 3). The increases in serum Lp-PLA2 levels at 2MPT were statistically significant.
Table 2. Periodontal and serum parameters at each study period: baseline (BL), 1 week after periodontal treatment (1WPT), and 2 months after the completion of periodontal treatment (2MPT) [median (minimum–maximum)].

<table>
<thead>
<tr>
<th>Periodontal parameters</th>
<th>C (n = 28)</th>
<th>HD (n = 29)</th>
<th>HS (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>1WPT</td>
<td>2MPT</td>
</tr>
<tr>
<td>PI</td>
<td>2.07 (0.68–3.00)</td>
<td>……………</td>
<td>0.35 (0.12–1.20)</td>
</tr>
<tr>
<td>GI</td>
<td>1.12 (0.35–2.58)</td>
<td>……………</td>
<td>0.23 (0.05–0.75)</td>
</tr>
<tr>
<td>PPD (mm)</td>
<td>2.74 (1.10–4.07)</td>
<td>……………</td>
<td>2.38 (1.96–3.50)</td>
</tr>
<tr>
<td>BOP (%)</td>
<td>50.02 (8.38–100)</td>
<td>……………</td>
<td>45.00 (0.23–100)</td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>3.17 (1.16–7.13)</td>
<td>……………</td>
<td>3.08 (2.06–5.60)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum parameters</th>
<th>C (n = 28)</th>
<th>HD (n = 29)</th>
<th>HS (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>1WPT</td>
<td>2MPT</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>3.51 (2.08–4.74)</td>
<td>3.90 (2.01–6.09)</td>
<td>3.87 (2.25–5.87)</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>164.00 (92.00–200.00)</td>
<td>170.00 (84.00–233.00)</td>
<td>176.00 (137.00–208.00)</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>96.50 (31.80–124.40)</td>
<td>97.50 (47.00–154.80)</td>
<td>98.60 (277.00–148.20)</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>48.00 (33.00–71.00)</td>
<td>45.50 (28.00–71.00)</td>
<td>45.00 (30.00–61.00)</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>17.40 (8.00–43.20)</td>
<td>21.90 (6.00–65.00)</td>
<td>23.20 (10.50–41.00)</td>
</tr>
<tr>
<td>TRG (mg/dL)</td>
<td>86.00 (40.00–206.00)</td>
<td>100.00 (30.00–325.00)</td>
<td>99.00 (45.00–207.00)</td>
</tr>
<tr>
<td>Lp-PLA2 (mmol min⁻¹ mL⁻¹)</td>
<td>8.56 (2.2–20.0)</td>
<td>7.01 (3.5–76.8)</td>
<td>8.20 (10.0–900.0)</td>
</tr>
<tr>
<td>hsCRP (mg/dL)</td>
<td>1.75 (0.01–6.82)</td>
<td>2.34 (0.05–7.82)</td>
<td>1.45 (0.02–4.92)</td>
</tr>
</tbody>
</table>
compared to baseline and 1WPT in the HS group (P = 0.016 and P = 0.009, respectively) (Table 3). There were no statistically significant differences in serum CRP levels among the study periods for all groups (P > 0.05).

4. Discussion

To the best of the authors' knowledge, this is the first study evaluating the role of periodontal therapy in serum levels of Lp-PLA2 and CRP in hyperlipidaemic patients with periodontitis. Serum parameters were assessed again at 1WPT and 2MPT and periodontal parameters were reevaluated at 2MPT because serum lipids and lipoproteins may be modified by acute phase reactants such as Lp-PLA2 and CRP associated with injury or inflammation (24,25). For the first period (1WPT), we aimed to evaluate the effects of the early phase of wound healing associated with mechanic instrumentation on serum inflammatory mediators.

Strict inclusion criteria were applied in this study in order to eradicate any systemic conditions that may play an important role in lipid metabolism and periodontal disease. A control group without treatment was not considered in the present study since it could give rise to some ethical problems regarding patients with serious metabolic disorders such as hyperlipidaemia. Furthermore, two different hyperlipidaemic groups (suggested diet only and diet with atorvastatin) were composed to evaluate the efficiency of periodontal therapy on the severity of the impaired lipid metabolism in this study.

Our previous studies indicated that inflammatory mediators might play a key role in the pathogenesis of both periodontal disease and hyperlipidaemia (14,15). However, the effects of periodontal therapy on the serum levels of lipoprotein-associated inflammatory mediators such as CRP and Lp-PLA2 were not investigated.

In this study, recruitment of patients was done by both a periodontist and a physician. Although the behavioural characteristics of the participants may affect the efficiency of the periodontal and antilipaemic treatments, the hyperlipidaemic patients declared that they complied with the physician's suggestions such as oral hygiene, physical activity, diet, and/or antilipaemic drug usage during the study period.

The present data showed decreases in the atherogenic lipid profile in the hyperlipidaemic groups for both study periods after the periodontal therapy. These findings are in line with those reported by Oz et al. (12) and Fentoğlu et al. (13), who showed beneficial effects of periodontal treatment on metabolic control of hypercholesterolaemic patients. Furthermore, these results are supported by some reports conducted in systemically healthy subjects with periodontitis (5,22,23).

In this study, there were insignificant increases in serum cholesterol levels in the systemically healthy group at 1WPT and 2MPT compared to the baseline. In fact, acute conditions lead to an increase in cholesterol production in humans (26). According to our results, there were also increases in TRG and VLDL levels at 1WPT compared to the baseline in all groups. In different types of acute events, plasma TRG levels might increase, remain stable, or decrease (27). Khovidhunkit et al. (28) reported that enhanced hepatic TRG results from the stimulation of lipolysis in peripheral and visceral adipose stores.

In this study, there were insignificant increases in serum Lp-PLA2 and CRP levels in the controls, who were systemically healthy individuals with levels of lipoprotein-associated inflammatory mediators such as CRP and Lp-PLA2 were not investigated.

Table 3. Statistically significant comparisons of periodontal and serum parameters of study groups according to study periods.

<table>
<thead>
<tr>
<th>Periodontal and serum parameters</th>
<th>C (n = 28)</th>
<th>HD (n = 29)</th>
<th>HS (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1WPT/BL</td>
<td>2MPT/1WPT</td>
<td>2MPT/BL</td>
</tr>
<tr>
<td>PI</td>
<td>N.A.</td>
<td>N.A.</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>GI</td>
<td>N.A.</td>
<td>N.A.</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>PPD (mm)</td>
<td>N.A.</td>
<td>N.A.</td>
<td>0.015*</td>
</tr>
<tr>
<td>BOP (%)</td>
<td>N.A.</td>
<td>N.A.</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>TRG (mg/dL)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>0.003*</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Lp-PLA2 (mmol min⁻¹ mL⁻¹)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ***P < 0.001. N.A. = not applicable, N.S. = not significant.

1WPT/BL = comparison of 1WPT to BL, 2MPT/1WPT = comparison of 2MPT to 1WPT, and 2MPT/BL = comparison of 2MPT to BL.
periodontitis, in both study periods. These findings are generally consistent with the previous studies conducted in normolipidaemic subjects with periodontitis (8). Periodontitis led to an increase in the plasma Lp-PLA2 levels in systemically healthy subjects with periodontitis (29). Moreover, periodontal treatment significantly reduced the serum activity of Lp-PLA2 in systemically healthy subjects with periodontitis (5).

According to the present results, the HD and HS groups had higher serum Lp-PLA2 and CRP levels compared to the C group at the baseline. However, the HS group had the lowest serum CRP levels at the baseline. This can be explained by the effects of statin therapy on serum Lp-PLA2 (30) and CRP levels (31). Some beneficial effects of statins on inflammatory mediators result from their nonlipid effects (31). Moreover, the combination of the periodontal therapy and antilipaemic treatment may provide beneficial effects for the metabolic and inflammatory control of hyperlipidaemia by decreasing proinflammatory cytokines (32).

In the present study, the HS group had higher BOP% values than the HD and C groups at baseline. The LDL content of Lp-PLA2 may be increased by acute inflammation (33). Considering the degree of impairment of the lipid metabolism in the HS group, the severity of periodontal inflammation and the degree of failure of the lipid metabolism may play important roles in the mutual relationship between periodontal disease and hyperlipidaemia.

In the C and HD groups, there were insignificant reductions in serum Lp-PLA2 and CRP levels at the end of both study periods. However, in the HS group, the significant increase in serum Lp-PLA2 is noteworthy at 2MPT compared to baseline and 1WPT. The effects of periodontal therapy on serum CRP levels in hyperlipidaemics who received statin were previously evaluated (13). In contrast to our earlier results (13), there were insignificant decreases in serum CRP levels in both hyperlipidaemic groups at both study periods in the present study. An antilipaemic therapy program including diet, statin usage, and physical activity may also provide additional benefits.

There is no correlation between Lp-PLA2 and CRP regarding the determination of the risk for cardiovascular disease (34). There was also no significant correlation between serum Lp-PLA2 and CRP levels in hyperlipidaemic patients with periodontal disease according to our previous results (15). CRP is transported freely in the plasma rather than bound to circulating lipoproteins (7). Therefore, it can be argued that serum Lp-PLA2 is more important than CRP, according to the present findings. In addition, the level of serum lipids as well as the tissue injury due to periodontal instrumentation may modify serum levels of Lp-PLA2 in hyperlipidaemics. Understanding the stages involved in the pathogenesis of Lp-PLA2 such as production, binding to circulating lipoproteins, and the amount of Lp-PLA2 remaining free will become more important for the hyperlipidaemic study population. Therefore, Lp-PLA2 can be a novel target for host modulation therapy (30,31).

Not only patients who had mild or moderate hyperlipidaemia (HD group) but also those with severe hyperlipidaemia (HS group) were included into this study because the aim was to evaluate the effects of periodontal therapy on the metabolic and inflammatory components of hyperlipidaemia as a metabolic disorder. Periodontal treatment led to a significant increase in serum Lp-PLA2 levels in the HS group at the end of this study period even though the HS group received statin therapy. In this context, the emphasis should be on the role of the intensity of the impairment of the lipid metabolism rather than the quantitative pathological serum lipid values in the association between inflammation (related to periodontitis) and hyperlipidaemia.

Finally, a combination of periodontal treatment and lipid-lowering therapy may lead to beneficial effects on the atherogenic lipid profile. However, the increases in serum Lp-PLA2 levels following periodontal treatment in hyperlipidaemics with severely impaired lipid metabolism may suggest that the application of conventional periodontal treatment can be combined with new therapeutic approaches including antimicrobial and/or antiinflammatory applications to prevent cardiovascular events in the hyperlipidaemic population. Further longitudinal studies in larger populations with different severities of both periodontitis and hyperlipidaemia are needed to clarify this association.

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