Is the relationship between periodontitis and hyperlipidemia mediated by lipoprotein-associated inflammatory mediators?

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ABSTRACT

Purpose: The aim of this study was to evaluate the serum levels of oxidized low-density lipoprotein (oxLDL), anti-oxLDL, and myeloperoxidase (MPO) in hyperlipidemic patients with periodontal disease.

Methods: This study included 123 patients with hyperlipidemia categorized based on metabolic control as mild to moderate (H1) (n=66) or poor (H2) (n=57), as well as systemically healthy controls (C) (n=68). Serum levels of lipids, oxLDL, anti-oxLDL, and MPO were evaluated, along with clinical periodontal parameters.

Results: The percentage of bleeding on probing (BOP%) and the clinical attachment level were significantly higher in the H2 group than in the C group. Patients with hyperlipidemia had a relatively high risk of developing periodontal disease. The oxLDL and anti-oxLDL levels were higher in H2 patients with periodontitis than in the control or H1 patients with periodontitis. In the H1 and H2 groups, the ratio of total cholesterol to high-density lipoprotein was significantly correlated with gingival index, BOP%, and oxLDL levels.

Conclusions: Our findings indicate that the lipoprotein-associated inflammatory mediators of oxLDL, anti-oxLDL, and MPO may play an important role in the relationship between periodontal disease and hyperlipidemia.

Keywords: Hyperlipidemias; Oxidized low density lipoprotein; Periodontal diseases; Peroxidase

INTRODUCTION

Atherosclerotic cardiovascular diseases (CVDs) are the leading cause of death globally, and hyperlipidemia is thought to contribute to more than 75% of cases [1]. Atherosclerosis, a pathological change associated with coronary heart disease, results from a lipoprotein-related inflammatory process [2] that also plays a key role in periodontal disease [3,4].

Lipoprotein oxidation has been demonstrated to be involved in atherosclerosis [5]. It has been reported that the immune response to infection and inflammation increases the serum concentration of oxidized lipids and stimulates low-density lipoprotein (LDL) oxidation [6].
Although Steinberg [7] described hypercholesterolemia and inflammation as “partners in crime,” the mechanisms of LDL oxidation are still subject to debate.

Oxidized LDL (oxLDL) is a biomarker that demonstrates the relationship between periodontal disease and atherosclerosis [8]. OxLDL is taken up by macrophages in the arterial wall, and it is involved in foam cell formation, stimulates inflammatory mediators, and contributes to the progression of atherosclerosis [9]. Myeloperoxidase (MPO) has come into prominence in various oxidation models [7,10], as the direct binding of MPO to LDL is known to be crucial to the development of atherosclerosis [11]. Anti-oxLDL is found in intimal lesions and circulating immune complexes, supporting the relationship between atherosclerotic and thrombotic events [12]. Increased anti-oxLDL levels have been reported in chronic diseases other than atherosclerosis, including periodontal disease [13].

The link between periodontitis and lipid metabolism disorders has recently become a focus of attention [14]. Pro-inflammatory cytokines and lipoprotein-associated inflammatory mediators have been repeatedly reported to be involved in periodontal disease [15] and hyperlipidemia [16,17]. We hypothesized that, in patients with hyperlipidemia, periodontal disease may affect the serum levels of oxLDL, anti-oxLDL, and MPO via systemic inflammation, and increased levels of those mediators in patients with hyperlipidemia and periodontal disease may contribute to the link between these diseases. As such, the purpose of the current study was to assess the serum levels of oxLDL, anti-oxLDL, and MPO in hyperlipidemic patients with periodontal disease.

MATERIALS AND METHODS

Study population
Sixty-eight systemically healthy patients (forming the control [C] group) from the Department of Periodontology and 123 hyperlipidemic patients from the Department of Internal Medicine at Suleyman Demirel University were recruited between January 2007 and April 2009. Hyperlipidemic patients were categorized as having either mild-to-moderately controlled hyperlipidemia (H1) or poorly controlled hyperlipidemia (H2). Informed consent was provided by each patient prior to the study. The ethics committee at Suleyman Demirel University (No. 09/11) approved the study in compliance with the Declaration of Helsinki.

The exclusion criteria were as follows: endocrine disorders, including diabetes mellitus, metabolic syndrome, and thyroid anomalies; chronic renal disease; treatment with lipid-lowering medications for longer than 1 month; ongoing hormone replacement therapy; a 3-fold elevation in liver enzymes above normal levels; treatment for periodontal disease within the past 6 months or treatment with antibiotics within the last 3 months; pregnant or lactating women; current or former smokers; and patients with fewer than 18 natural teeth.

Periodontal examination and study groups
Plaque index [18], gingival index (GI) [19], and percentage of bleeding on probing (BOP%) were measured at 4 sites (mesial, buccal, and distal on the facial surface and buccal on the lingual surface), while probing pocket depth (PD) and clinical attachment loss (CAL) were measured at 6 sites (mesial, buccal, and distal on both the facial and lingual surfaces) in the whole dentition excluding wisdom teeth by a periodontist (O. F.) using a periodontal probe (Williams, Hu-Friedy, Chicago, IL, USA). Intra-examiner reliability was evaluated with...
2 repeated measurements at 1-hour intervals in 5 patients. Intra-examiner weighted kappa values (±1 mm) ranged from 0.86 to 0.92 for PD and from 0.76 to 0.80 for CAL. The intra-rater correlation was calculated for each parameter, yielding values of 0.91 and 0.93 for PD and CAL, respectively.

The groups were also categorized into 3 subgroups according to periodontal status: gingival health (h), <10% BOP with probing depths ≤3 mm; gingivitis (g), ≥10% BOP with probing depths ≤3 mm; and periodontitis (p), detectable interdental CAL at ≥2 non-adjacent teeth or detectable buccal or oral CAL ≥3 mm with pocketing >3 mm at ≥2 teeth [20,21]. The stage and grade of the periodontitis were also assessed [21]. None of the patients had periodontitis of grade b or c. Therefore, 9 groups were formed as follows: Ch (n=16), Cg (n=24), Cp (n=28), H1h (n=14), H1g (n=23), H1p (n=29), H2h (n=14), H2g (n=20), and H2p (n=23).

**Serum parameters and laboratory analyses**

Approximately 5 mL of blood was collected and centrifuged at 4,000 rpm for 4 minutes. Serum samples were obtained and stored at −80°C until testing to detect levels of triglycerides (TRG), total cholesterol (TC), LDL, high-density lipoprotein (HDL), very LDL (VLDL), oxLDL, anti-oxLDL, and MPO. The following values were used to indicate pathological lipid levels: TRG >200 mg/dL, TC >200 mg/dL, LDL >130 mg/dL, HDL <35 mg/dL, and VLDL >40 mg/dL [22]. Patients in the H1 group had plasma LDL values <160 mg/dL and >130 mg/dL, while patients in the H2 group had plasma LDL values >160 mg/dL. Serum oxLDL, anti-oxLDL, and MPO levels were evaluated via enzyme-linked immunosorbent assay using commercially available kits (Biomedica Medizinprodukte GmbH, Vienna, Austria).

**Statistical analyses**

A statistical package program (SPSS version 11.0, SPSS Inc., Chicago, IL, USA) was used to analyze the data. The normality of the distribution was evaluated with the Kolmogorov-Smirnov test. Differences between the study groups were assessed using the Kruskal-Wallis test followed by the Mann-Whitney U test. Analysis of variance was performed, and Spearman correlation analysis was used to compare the serum and periodontal parameters. The relationships between independent variables (serum parameters) and dependent variables (periodontal parameters), adjusted for confounders (age, sex, body mass index [kg/m²], tooth brushing frequency, and socioeconomic status), were evaluated using multiple logistic regression analysis. The a posteriori power of this study was found to be greater than 85% (NCSS/PASS 2000, Dawson Edition, NCSS Statistical Software, Kaysville, UT, USA) in the evaluation of variations in the clinical and serum parameters among the groups and subgroups.

**RESULTS**

Sixty-six hyperlipidemic patients aged 30 to 57 years (36 female and 30 male) with mild-to-moderately control, 57 hyperlipidemic patients aged 35 to 57 (27 female and 30 male) with poor metabolic control, and 68 systemically healthy controls aged 31 to 54 years (37 female and 31 male) were included in this study. No significant differences were observed among the groups with regard to age, sex, tooth brushing frequency, or socioeconomic factors (P>0.05) (Table 1).

Fifteen patients in the H2 group used atorvastatin (10 mg). The number of patients with hypertension was similar between the H1 and H2 groups. Body mass index values ranged from 16.7 kg/m² to 43.9 kg/m² and from 17.8 kg/m² to 38.2 kg/m² for the hyperlipidemic
groups and the systemically healthy control group, respectively. The severity of periodontitis was similar among the groups (Table 1).

The periodontal and serum parameters of the groups are shown in Tables 2-4. BOP%, CAL, oxLDL, and anti-oxLDL levels were elevated in the H2 group relative to the C group (P<0.001)

Table 1. Demographic characteristics

| Variable                        | C (n=68)          | H1 (n=66)         | H2 (n=57)         | P value |
|---------------------------------|-------------------|-------------------|-------------------|---------|
| Age (yr)                        | 37.18±1.50        | 45.23±1.37        | 49.62±1.32        | 0.396   |
| Sex Male                        | 31                | 30                | 30                |         |
| Sex Female                      | 37                | 36                | 27                |         |
| BMI (kg/m²)                     | 25.65 (17.8–38.2) | 28.24 (23.3–39.6) | 28.66 (16.7–43.9) | 0.096   |
| Hypertension                    | NA                | 6                 | 8                 |         |
| Statin users                    | NA                | 0                 | 15                |         |
| Tooth brushing frequency       |                   |                   |                   |         |
| Never                           | 26                | 26                | 20                |         |
| Irregular                       | 20                | 20                | 18                |         |
| ≥1 time(s) per day             | 22                | 20                | 19                |         |
| Socioeconomic variables        |                   |                   |                   |         |
| Education level                |                   |                   |                   |         |
| Elementary school              | 41                | 41                | 34                |         |
| High school                     | 27                | 25                | 23                |         |
| Employment status              |                   |                   |                   |         |
| Employed                        | 40                | 40                | 33                |         |
| Unemployed                      | 7                 | 6                 | 5                 |         |
| Retired                         | 21                | 20                | 19                |         |
| Gingival health                 |                   |                   |                   |         |
| Gingivitis                      | 16                | 14                | 14                |         |
| Gingivitis                      | 24                | 23                | 20                |         |
| Severity of periodontitis       |                   |                   |                   |         |
| Stage I                         | 15                | 15                | 12                |         |
| Stage II                        | 12                | 14                | 11                |         |
| Stage III                       | 1                 | NA                | NA                |         |
| Stage IV                        | NA                | NA                | NA                |         |

Data are shown as mean±standard error, mean (range), or number (%).
C: systemically healthy control, H1: hyperlipidemic patients with mild-to-moderate metabolic control, H2: hyperlipidemic patients with poor metabolic control, BMI: body mass index, NA: not applicable.

Table 2. Periodontal and serum parameters of the main groups

| Variable               | C (n=68)          | H1 (n=66)         | H2 (n=57)         | P value |
|------------------------|-------------------|-------------------|-------------------|---------|
| PI                     | 1.42 (0.75–2.15)  | 1.23 (0.78–1.85)  | 1.29 (0.71–2.03)  | 0.396   |
| GI                     | 0.77 (0.52–1.23)  | 0.87 (0.56–1.16)  | 0.92 (0.67–1.22)  | 0.551   |
| PD (mm)                | 2.15 (1.77–2.85)  | 2.10 (1.70–2.70)  | 2.34 (2.08–2.90)  | 0.096   |
| BOP%                   | 38.00 (24.00–56.66) | 40.66 (25.85–62.89) | 46.42 (20.25–90.16) | 0.006   |
| CAL (mg/dL)            | 2.77 (1.75–2.90)  | 2.24 (1.73–2.90)  | 2.95 (2.06–3.29)  | 0.008   |
| TC (mg/dL)             | 159.50 (142.00–178.75) | 216.00 (206.00–229.00) | 225.00 (206.00–251.50) | 0.000   |
| LDL (mg/dL)            | 94.00 (74.30–105.70) | 144.80 (125.00–157.00) | 143.00 (124.60–169.50) | 0.000   |
| HDL (mg/dL)            | 45.00 (38.00–50.00) | 42.00 (38.00–48.00) | 47.00 (41.00–55.00) | 0.006   |
| TC/HDL                 | 3.64 (2.95–4.16)  | 5.11 (4.62–5.86)  | 5.50 (3.87–5.64)  | 0.000   |
| VLDL (mg/dL)           | 19.40 (15.25–24.45) | 29.80 (21.00–40.00) | 26.00 (20.10–49.00) | 0.000   |
| TRG (mg/dL)            | 97.00 (88.25–128.25) | 136.00 (103.00–183.00) | 127.00 (102.50–186.50) | 0.000   |
| MPO (ng/mL)            | 56.56 (32.64–80.32) | 59.52 (32.40–82.54) | 56.71 (29.52–79.72) | 0.210   |
| oxLDL (ng/mL)          | 155.32 (113.42–202.96) | 166.97 (116.95–302.77) | 224.12 (133.04–397.54) | 0.016   |
| Anti-oxLDL (mU/mL)     | 32.91 (14.74–55.72) | 37.92 (15.14–55.72) | 45.27 (20.23–49.11) | 0.015   |

Data are shown as median (25%–75%).
C: systemically healthy control, H1: hyperlipidemic patients with mild-to-moderate metabolic control, H2: hyperlipidemic patients with poor metabolic control, PI: plaque index, GI: gingival index, PD: probing pocket depth, BOP: bleeding on probing, CAL: clinical attachment loss, TC: total cholesterol, LDL: low-density lipoprotein, HDL: high-density lipoprotein, TC/HDL: ratio of total cholesterol to high-density lipoprotein, VLDL: very low-density lipoprotein, TRG: triglyceride, MPO: myeloperoxidase, oxLDL: oxidized low density lipoprotein, anti-oxLDL: anti-oxidized low density lipoprotein.

*Significant difference from the C group (P<0.05); **Significant difference from the H1 group (P<0.05).
Table 3. Periodontal parameters of the subgroups

| Variable | Ch (n=16) | Cg (n=24) | Cp (n=28) | H1h (n=14) | H1p (n=29) | H2h (n=14) | H2g (n=20) | H2p (n=23) |
|----------|-----------|-----------|-----------|-------------|------------|-------------|------------|------------|
| PI       | 1.09 (0.10–2.75) | 1.31 (0.15–3.00) | 2.07 (0.68–3.00) | 1.17 (0.16–2.58) | 1.51 (0.36–2.65) | 1.17 (0.30–2.89) | 0.98 (0.21–2.82) | 1.00 (0.18–2.56) |
| GI       | 0.54 (0.12–1.67) | 0.67 (0.20–1.45) | 1.12 (0.35–2.58) | 0.56 (0.21–2.43) | 0.92 (0.44–1.57) | 0.91 (0.33–2.21) | 0.83 (0.34–1.28) | 1.00 (0.50–2.54) |
| PD (mm)  | 1.90 (1.00–2.56) | 2.00 (1.02–2.52) | 2.74 (1.10–4.07) | 1.73 (1.00–2.40) | 2.00 (1.02-2.64) | 1.76 (1.03–2.52) | 2.11 (1.69–3.00) | 2.24 (1.77–3.87) |
| BOP%     | 7.91 (1.66–9.05) | 32.50 (15.33–100) | 50.02 (8.38–100) | 9.85 (7.00–9.20) | 33.30 (7.28–100) | 52.18 (2.38–9.80) | 21.74 (17.13–66.66) | 49.50 (6.00–100) |
| CAL (mm) | 1.90 (0.10–2.56) | 2.00 (0.12–2.52) | 3.17 (1.16–7.13) | 1.73 (0.00–2.40) | 2.68 (1.02–2.94) | 3.15 (1.25–4.38) | 1.92 (1.03–2.52) | 2.84 (1.69–4.00) |

Data are shown as median (25%-75%).

Ch: systemically healthy control and gingival health, Cg: systemically healthy control and gingivitis, Cp: systemically healthy control and periodontitis, H1h: hyperlipidemic patients with mild-to-moderate metabolic control and periodontitis, H1p: hyperlipidemic patients with mild-to-moderate metabolic control and gingivitis, H2h: hyperlipidemic patients with mild-to-moderate metabolic control and gingivitis, H2g: hyperlipidemic patients with poor metabolic control and periodontitis, H2p: hyperlipidemic patients with poor metabolic control and gingivitis, PI: plaque index, GI: gingival index, PD: probing pocket depth, BOP: bleeding on probing, CAL: clinical attachment loss.

Significant difference from the Ch group (P<0.05); Significant difference from the Cg group (P<0.05); Significant difference from the Cp group (P<0.05); Significant difference from the H1h group (P<0.05); Significant difference from the H1g group (P<0.05); Significant difference from the H2h group (P<0.05); Significant difference from the H2g group (P<0.05).

Table 4. Serum parameters of the subgroups

| Variable | Ch (n=16) | Cg (n=24) | Cp (n=28) | H1h (n=14) | H1p (n=29) | H2h (n=14) | H2g (n=20) | H2p (n=23) |
|----------|-----------|-----------|-----------|-------------|------------|-------------|------------|------------|
| TC (mg/dL) | 146.50 (113–191) | 170.00 (140.00–197.00) | 164.00 (90.00–200.00) | 207.00 (76–241) | 221.00 (202.00–249.00) | 220.50 (209.00–231.00) | 223.00 (165.00–290.00) | 215.00 (155–301) |
| LDL (mg/dL) | 73.50 (52.40–126.40) | 66.00 (66.00–124.60) | 110.40 (31.80–124.40) | 97.00 (105.00–168.00) | 150.80 (99.00–192.80) | 150.00 (73.60–263.20) | 150.00 (82.00–189.60) | 179.00 (78.00–216.00) |
| HDL (mg/dL) | 45.50 (32.00–59.00) | 41.00 (30.00–62.00) | 48.00 (33.00–71.00) | 40.00 (30.00–69.00) | 40.00 (30.00–62.00) | 40.00 (30.00–69.00) | 40.00 (30.00–69.00) | 40.00 (30.00–69.00) |
| TRG (mg/dL) | 110.00 (9.80–54.00) | 110.00 (14.00–41.00) | 80.00 (8.00–43.20) | 149.00 (13.00–99.40) | 142.00 (62.00–174.00) | 150.00 (62.00–174.00) | 150.00 (62.00–174.00) | 150.00 (62.00–174.00) |
| MPO (mg/L) | 44.00 (8.79–146.40) | 38.02 (14.59–118.88) | 76.46 (12.89–142.20) | 47.82 (10.51–95.56) | 47.82 (8.22–136.69) | 47.82 (8.22–136.69) | 47.82 (8.22–136.69) | 47.82 (8.22–136.69) |
| OxLDL (mg/L) | 156.03 (97.69–299.32) | 199.55 (472.00–439.80) | 132.04 (15.45–351.34) | 192.97 (70.22–6849.97) | 213.46 (51.57–666.95) | 213.46 (51.57–666.95) | 213.46 (51.57–666.95) | 213.46 (51.57–666.95) |
| Anti-oxLDL (mU/mL) | 49.01 (6.26–203.35) | 49.01 (6.26–203.35) | 21.80 (4.84–215.31) | 21.80 (4.84–215.31) | 21.80 (4.84–215.31) | 21.80 (4.84–215.31) | 21.80 (4.84–215.31) | 21.80 (4.84–215.31) |

Data are shown as median (25%-75%).

Ch: systemically healthy control and gingival health, Cg: systemically healthy control and gingivitis, Cp: systemically healthy control and periodontitis, H1h: hyperlipidemic patients with mild-to-moderate metabolic control and periodontitis, H1p: hyperlipidemic patients with mild-to-moderate metabolic control and gingivitis, H2h: hyperlipidemic patients with mild-to-moderate metabolic control and gingivitis, H2g: hyperlipidemic patients with poor metabolic control and periodontitis, TC: total cholesterol, LDL: low-density lipoprotein, HDL: high-density lipoprotein, TC/HDL: ratio of total cholesterol to high-density lipoprotein, VLDL: very low-density lipoprotein, TRG: triglyceride, MPO: myeloperoxidase, oxLDL: oxidized low-density lipoprotein, anti-oxLDL: anti-oxidized low-density lipoprotein.

Significant difference from the Ch group (P<0.05); Significant difference from the Cg group (P<0.05); Significant difference from the Cp group (P<0.05); Significant difference from the H1h group (P<0.05); Significant difference from the H1g group (P<0.05); Significant difference from the H2h group (P<0.05); Significant difference from the H2g group (P<0.05).
GI, PD, BOP%, and CAL were higher in the H2g group than in the H1g and Cg groups. BOP% was significantly higher in the H2p group than in the Cp and H1p groups ($P<0.001$) (Table 3). The oxLDL and anti-oxLDL levels were also elevated in the H2p group compared with the Cp and H1p groups ($P<0.05$) (Table 4).

Hyperlipidemia was found to have an odds ratio (OR) of 2.48 ($P=0.019$; 95% confidence interval [CI], 0.63–9.76) with regard to the development of periodontal disease (Table 5). In both hyperlipidemic groups, the TC/HDL ratio was significantly correlated with GI, BOP%, and oxLDL levels ($P<0.05$). CAL values were positively associated with the serum MPO and anti-oxLDL levels in the hyperlipidemic groups ($P<0.05$). A significant positive association was also observed between BOP% and anti-oxLDL in the H2 group, and a significant positive correlation was found between the severity of periodontitis and both TC/HDL and anti-oxLDL levels in the hyperlipidemic groups (Table 6).

### Table 5. OR for assessments of serum parameters on the development of periodontal disease

| Serum parameter | Model | P value | Crude OR (95% CI) |
|-----------------|-------|---------|------------------|
| TC (mg/dL)      | 0.180 | 0.97 (0.94–1.01) |
| LDL (mg/dL)     | 0.460 | 1.01 (0.98–1.03) |
| HDL (mg/dL)     | 0.020 | 2.48 (0.63–9.76) |
| VLDL (mg/dL)    | 0.500 | 0.99 (0.97–1.02) |
| TRG (mg/dL)     | 0.450 | 1.00 (1.00–1.10) |
| MPO (ng/mL)     | 0.040 | 1.00 (1.00–1.02) |
| oxLDL (ng/mL)   | 0.260 | 1.00 (1.00–1.00) |
| Anti-oxLDL (mU/mL) | 0.490 | 1.00 (0.99–1.01) |

OR: odds ratio, CI: confidence interval, TC: total cholesterol, LDL: low-density lipoprotein, HDL: high-density lipoprotein, TC/HDL: ratio of total cholesterol to high-density lipoprotein, VLDL: very low-density lipoprotein, TRG: triglyceride, MPO: myeloperoxidase, oxLDL: oxidized low-density lipoprotein, anti-oxLDL: anti-oxidized low-density lipoprotein.

### Table 6. Significant correlations between clinical periodontal parameters and serum parameters

| Parameter | C (n=68) | H1 (n=66) | H2 (n=57) |
|-----------|---------|-----------|-----------|
|           | $\rho$  | P value   | $\rho$  | P value   | $\rho$  | P value   |
| PI and MPO | 0.476  | 0.000     | -       | -         | -       | -         |
| GI and oxLDL | 0.263  | 0.030     | -       | -         | -       | -         |
| BOP% and oxLDL | 0.471  | 0.000     | -       | -         | -       | -         |
| PD and anti-oxLDL | -    | -         | 0.255  | 0.044     | -       | -         |
| CAL and MPO | -      | -         | 0.339  | 0.007     | 0.409   | 0.002     |
| CAL and anti-oxLDL | -    | -         | 0.279  | 0.027     | 0.280   | 0.037     |
| GI and TC/HDL | -     | -         | 0.565  | 0.015     | 0.302   | 0.022     |
| BOP% and TC/HDL | -    | -         | 0.280  | 0.034     | 0.268   | 0.036     |
| PD and HDL | -       | -         | -0.565 | 0.015     | -       | -         |
| GI and LDL | -       | -         | -       | -         | 0.352   | 0.007     |
| GI and VLDL | -      | -         | -       | -         | 0.282   | 0.034     |
| MPO and oxLDL | -    | -         | -       | -         | 0.264   | 0.047     |
| TC/HDL and oxLDL | -    | -         | -       | -         | 0.454   | 0.001     |
| Periodontitis severity and TC/HDL | -    | -         | 0.277  | 0.037     | 0.678   | 0.002     |
| Periodontitis severity and anti-oxLDL | -    | -         | 0.216  | 0.034     | 0.578   | 0.015     |

C: systemically healthy control, H1: hyperlipidemic patients with mild-to-moderate metabolic control, H2: hyperlipidemic patients with poor metabolic control, $\rho$: Spearman’s correlation coefficient, PI: plaque index, GI: gingival index, PD: probing pocket depth, BOP%: percentage of bleeding on probing, CAL: clinical attachment loss, LDL: low-density lipoprotein, HDL: high-density lipoprotein, TC/HDL: ratio of total cholesterol to high-density lipoprotein, VLDL: very low-density lipoprotein, MPO: myeloperoxidase, oxLDL: oxidized low-density lipoprotein, anti-oxLDL: anti-oxidized low-density lipoprotein.
The partial standardized regression coefficients of the variables included in the model following multiple regression analysis are presented in Table 7. In the hyperlipidemic groups, TC/HDL was positively correlated with BOP%. CAL was also found to be associated with serum MPO and anti-oxLDL levels after further analyses in the hyperlipidemic groups.

DISCUSSION

To the best of our knowledge, no study has evaluated serum oxLDL, anti-oxLDL, and MPO levels in hyperlipidemic patients with periodontal disease. Particularly in the context of the impact of bacterial lipopolysaccharide-LDL interactions on lipoprotein metabolism [23], the potential link between periodontal disease and hyperlipidemia may be very important.

One issue that must be emphasized is the rigidity of the inclusion criteria of the present study. To evaluate systemic status, all participants were subjected to detailed medical evaluations using biochemical analyses, rather than utilizing the subjects’ own statements. Also, all of the individuals were recruited from a single center. Thus, both the similarities among the groups regarding sociodemographics and the exclusion of current and ex-smokers may be important factors that strengthen this study.

In this study, hyperlipidemic individuals with poor metabolic control showed higher BOP% and CAL than did systemically healthy individuals (Table 2). These findings are supported by investigations conducted in hyperlipidemic populations [16,17]. Our results also suggested that GI and BOP% were significantly associated with the TC/HDL ratio in the hyperlipidemic groups (Table 6). The discussion of our results is based on the TC/HDL ratio because this ratio has been reported to be relatively predictive of CVD [24]. Maglakelidze et al. [25] reported that hypercholesterolemia damages the basal membrane and influences permeability. Thus, regarding pathogenesis, hypercholesterolemia could be considered a risk factor for periodontal disease. Several studies have confirmed that patients with hypercholesterolemia have higher values for parameters associated with periodontal disease than systemically healthy controls [16,17]. Additionally, systemically healthy individuals with periodontitis have higher serum lipid levels than periodontally healthy controls [26].

Clinical studies that examine the association among the serum oxLDL, anti-oxLDL, and MPO levels and periodontal disease are generally performed in individuals with periodontitis [27-29]. Consequently, both categorization of metabolic control of hyperlipidemia by a physician and determination of periodontal subgroups are required to clarify the association between periodontal disease and hyperlipidemia with regard to serum levels of oxLDL, anti-oxLDL, and MPO.

Table 7. Multiple regression analysis of systemic and periodontal parameters in the hyperlipidemic groups

| Variables       | H1 (n=66) |          | H2 (n=57) |          |
|-----------------|-----------|----------|-----------|----------|
|                 | BOP% (β) | CAL (β)  | BOP% (β) | CAL (β)  |
| TC/HDL          | 0.310a   | NA       | 0.396a   | NA       |
| MPO             | NA       | 0.323a   | NA       | 0.349a   |
| Anti-oxLDL      | NA       | 0.190a   | 0.296a   | 0.242a   |

H1: hyperlipidemic patients with mild-to-moderate metabolic control, H2: hyperlipidemic patients with poor metabolic control, BOP%: percentage of bleeding on probing, CAL: clinical attachment loss, β: partial standardized regression coefficient, TC/HDL: ratio of total cholesterol to high-density lipoprotein, MPO: myeloperoxidase, anti-oxLDL: anti-oxidized low-density lipoprotein, NA: not applicable.

asterisk Statistical significance (P<0.05).
In the present study, TC/HDL ratio was positively correlated with GI, BOP%, and oxLDL in the H1 and H2 groups. BOP% and CAL were shown to be associated with anti-oxLDL in the H2 group after adjusting for confounders (Table 7). Furthermore, periodontitis severity as evidenced by stage (Table 1) was also positively correlated with both TC/HDL ratio and anti-oxLDL level (Table 6). Our results emphasize the novel point that both periodontal disease and impaired lipid metabolism may be very important in the pathogenesis of inflammatory pathways. Thus, increased serum levels of oxLDL and anti-oxLDL related to periodontal disease may lead to an increase in periodontal destruction or atheromatous lesions associated with the hyper-responsive phenotype in patients with a high serum lipid profile.

According to our findings, CAL was significantly correlated with MPO in the hyperlipidemic groups (Tables 6 and 7). MPO is an antimicrobial enzyme that is found in polymorphonuclear leukocytes and that plays a crucial role in periodontal disease-associated inflammation [30]. The protective role of polymorphonuclear leukocytes in the early response to periodontal infection [31] enhances the importance of dietary lipids in periodontal pathogenesis. Additionally, hyperlipidemia may play a much more meaningful role than hyperglycemia in the existence of the hyper-responsive monocytic phenotype [32] and in the development of diabetic complications [33]. Therefore, hyperlipidemia may be important in the pathogenesis of periodontal disease associated with increased neutrophil function.

In our study, MPO level was shown to be correlated with oxLDL levels in the H2 group. This result has been corroborated by researchers [34,35] who reported the importance of MPO in the oxidation of LDL. As stated above, no data currently exist regarding the serum levels of MPO, oxLDL, and anti-oxLDL in hyperlipidemic patients with periodontal disease. In the current study, the H2p group had higher oxLDL and anti-oxLDL levels than the Cp group (Table 4). Our results are supported by data emphasizing that MPO-derived oxidants can cause the tissue damage observed in chronic inflammation [36]. With regard to serum anti-oxLDL levels, our findings have also been confirmed by other studies that suggested that anti-oxLDL can be used as a marker for several diseases [12]. It has also been reported that the decrease in the anti-oxLDL level after the administration of fenofibrate and vitamin E was gradual and persisted for many months; this parameter is therefore considered suitable for the description of chronic inflammation in an organism [12].

Although periodontitis and gingivitis share similar initial pathogenesis, those diseases have different clinical signs [37]. Thus, in this present study, subgroups were delineated according to periodontal status. In our study, the H2g subgroup had higher GI, PD, BOP%, and CAL than the Cg subgroup, and higher BOP% values were observed in the H2p subgroup than in the Cp and H1p subgroups. Moreover, the highest values of BOP% and CAL were seen in the H2p subgroup (Table 3). The H2p subgroup had higher oxLDL and anti-oxLDL levels than the Cp subgroup (Table 4). It is necessary to emphasize the role of inflammation-mediated periodontal destruction in the bidirectional relationship between periodontal disease and hyperlipidemia.

Although we adjusted for certain confounders, the contribution of diet and/or statin therapy, genetics, or other lifestyle habits (i.e., diet or physical activity) on both periodontal destruction and poor hyperlipidemic control should not be ignored. In fact, based on their anti-inflammatory properties, statins and a low-fat diet serve a protective function in periodontal tissues and in artery walls [38,39]. Both the severity of periodontal disease and
the degree of impairment of lipid metabolism could have affected the findings regarding the association between lipoprotein-associated inflammatory mediators and periodontal disease.

In this study, patients with hyperlipidemia had a higher risk of periodontal disease in terms of the TC/HDL ratio ($P=0.019; \text{OR, } 2.48; 95\% \text{ CI, } 0.63–9.76$) (Table 5). Our findings may provide important support to existing literature that suggests a link between these diseases [16,17]. However, we could only confirm an association between these factors and outcomes. Although cohort studies are recommended to confirm the bidirectional connection between periodontal disease and hyperlipidemia, the examination of hyperlipidemic patients with different levels of metabolic control in this study may still be noteworthy.

In conclusion, the TC/HDL ratio was found to be significantly correlated with GI, BOP%, and oxLDL levels in the hyperlipidemic groups. Significant correlations were also found between MPO and oxLDL levels in hyperlipidemic patients with poor metabolic control. Additionally, MPO and anti-oxLDL levels were also significantly associated with CAL in the hyperlipidemic groups after further analysis. Therefore, the increases in serum lipid, MPO, oxLDL, and/or anti-oxLDL levels may modify inflammatory events that are associated with both periodontal disease and hyperlipidemia. Further studies are needed to clarify the mechanisms behind the relationship between periodontal disease and hyperlipidemia.

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